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VOLUME 9 OCTOBER, 1933 NUMBER 4

SEED CONTENT, SEEDLING PRODUCTION AND FRUITFULNESS IN APPLES[1]

BY W. H. BRITTAIN[2] AND C. C. EIDT[3]

Abstract

The behavior of different types of apple crosses was found to be very similar, whether or not emasculation was practiced, indicating that selfing was not a serious factor in the latter case. In both cases it was found that (1) all varieties were fruitful when diploid varieties were used as male parents, though triploid × triploid crosses sometimes approximated the latter in this respect, and that (2) all varieties, regardless of chromosome constitution, were less fruitful when triploids were used as male parents. Though triploid varieties had a lower seed content than diploids, the seed content within the variety was characteristically greater wherever diploids were used as pollen parents. The results obtained with respect to seedlings were in general agreement with those based on seed alone, the order of production being as follows: first, diploid × diploid; second, triploid × diploid; third, diploid × triploid; and fourth, triploid × triploid. No correlation between seed content and weight could be demonstrated in Gravenstein, Baldwin, King and Wagener and a barely significant correlation in the case of Northern Spy. Various morphological abnormalities resulting from imperfect pollination are described.

Introduction

A number of workers have pointed out the relation that exists between development of seed and the measure of success of the cross. Some data from experiments on the production of viable seedlings are also available, though most of these experiments have been made from the breeding standpoint. Bach (1) has noted that the number of seeds in an apple is definitely influenced by the pollen parent. Musser and Andrews (19) report that the number of seeds per apple is a good index of the value of the pollen parent; Burrell and Parker (3) found the relative value of various pollen varieties on McIntosh to be indicated by seed counts. That the number of viable seeds is generally lower in fruits resulting from selfing has been stated by Johansson (13). Crandall (4) showed that self-pollinations were generally unsatisfactory both in the number of fruit produced and in the number and vigor of seedlings

[1] *Manuscript received July 6, 1933.*
The data in this paper were obtained in part in the course of an investigation of the pollination of the apple, carried out under the chairmanship of Mr. Arthur Gibson, Dominion Entomologist, and in part in the course of an apple breeding investigation conducted as part of the regular work of the Dominion Experimental Station, Kentville, Nova Scotia.

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resulting from such self-pollinations. Howlett (11) working with Stayman Winesap found no significant difference between the seed content of fruit produced by various pollinizers. The same worker was also unable to find any significant difference in seed germination between effective and ineffective pollinizers. Latimer (15) found a notable difference in the ability of different pollens to cause seeds to develop in McIntosh. Crane and Lawrence (5) state that often a single seed is sufficient for development in apples and that even it may be imperfect and therefore fruitfulness may be obtained in spite of a high degree of generational sterility.

According to many other workers imperfectly fertilized apples tend to be one-sided and several have presented evidence to show that there is a correlation between weight and number of seeds (Müller-Thurgau (18); Ewert (7); Heinicke (10); Kobel (14); Latimer (15, 16); Einset (6). Einset found a coefficient of correlation amounting to 0.237 ± 0.059 between weight of fruit and number of filled seeds in Gravenstein and of 0.349 ± 0.055 when all seeds, empty and filled, were considered. His calculations were based on only 118 fruits.

It may be observed that of all commercial varieties studied Gravenstein would be least likely to show such a correlation, since this variety gave the smallest average number of seeds and the largest percentage of seedless apples. Further evidence on this point is presented later. Ewert (8, 9), in a consideration of the subject of parthenocarpy in apples, contends that the parthenocarpic development of fruit can be encouraged by an abundance of available food. By preventing all the blossoms from being pollinated he was able to produce seedless fruit as large as normal specimens.

Kobel (14) has noted the tendency of varieties with a low pollen germination and irregular chromosome distribution to produce few or no seeds. He describes as "false parthenocarpy" the condition that obtains when fertilization has actually taken place, but, owing to the irregular chromosome distribution, further seed development does not occur.

It has been pointed out by Einset (*loc. cit.*) and also by other workers (Heinicke (10), MacDaniels and Heinicke (20)) that normal seed production is so intimately connected with the physiological processes of the fruit, that apples with developed seeds have an advantage in the competition for water and organic nutrients over those with fewer or no seeds, in that the presence of seeds is necessary for the normal development of the conducting tissue in the fruit that connects it with the tree.

The foregoing review, though incomplete, will serve to give an indication of the present status of opinion with respect to the problem that forms the subject of this paper.

Normal Seed Content of Standard Varieties

The type number of embryos in the apple fruit is ten, though with Northern Spy, Deacon Jones, and possibly other varieties, double this number appears

to be typical. Few individual apples, however, produce the maximum number of seeds and the average is far below this figure. The following table shows the average number of seeds produced by four standard varieties over a period of three years, the figures being based on a count of 500 drop apples and 200 picked apples of each variety for each of the three years. Drop apples refer to those that come off in the so-called "June drop," though "July drop" would be more descriptive of the situation in Nova Scotia.

TABLE I

AVERAGE NUMBER OF SEEDS* IN DIFFERENT VARIETIES BASED ON COUNTS FROM 16 ORCHARDS

Variety	1928		1929		1930		Average	
	Drop	Picked	Drop	Picked	Drop	Picked	Drop	Picked
Gravenstein	2.22	3.75	1.98	3.19	2.54	3.60	2.24	3.35
King	4.15	5.60	3.55	5.40	3.93	5.99	3.85	5.55
Baldwin	3.80	5.45	3.53	5.54	4.45	5.78	3.96	5.62
Spy	5.61	8.04	5.53	8.58	5.90	8.36	5.67	8.37

**Seed refers to total seed whether filled or not.*

It will be seen that the results are reasonably uniform, Gravenstein producing fruit with the smallest number of seeds, Spy the largest. In all varieties the average number of seeds in the drop fruit is less than in the picked, though individual drop fruit sometimes have more seeds than certain individual picked fruit. It is worthy of note that the first three varieties are triploid forms having 51 instead of the normal 34 chromosomes, and are typically few-seeded.

In the case of tented Gravensteins unsupplied with bees it was possible to produce seedless fruits as large as or even larger than normal ones, but the number so produced was less than where a satisfactory pollinizer and bees were supplied and in no case was a commercial crop of apples secured. Perfectly formed Gravensteins, as large as or larger than the average, containing no seeds whatever are commonly found in commercial orchards. Gravenstein is the only variety tested by the writers that showed this tendency, though others have been recorded. However, many of these seedless Gravensteins are abnormal in other respects as will be explained elsewhere. It is interesting to contrast the behavior of another triploid, *viz.*, Baldwin, which rarely, if ever, produces seedless fruit and which, when selfed, produces an average of over three seeds per fruit. The fact that Baldwin is self-compatible, whereas Gravenstein is quite self-incompatible may, in conjunction with triploidy, explain the foregoing fact. On the other hand, such varieties as Spy, tented under the same conditions, sometimes produced no fruit and, in all cases, a very small percentage set was secured, while seedless apples were not found in these tests.

The following tables, showing the seed content of certain standard varieties with both diploids and triploids used as male parents, have been compiled

from results of our pollination studies. These tables are based on four years results and are presented to show the massed results from these two types of crosses. More detailed varietal analyses are presented later.

TABLE II

AVERAGE NUMBER OF SEEDS OF STANDARD VARIETIES WHEN DIPLOIDS ARE USED AS MALE PARENTS

Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit	Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit
Gravenstein	16959	1739	3.34	Baldwin	14585	1433	5.31
King	16132	527	5.35	Spy	14971	1698	9.38
Golden Russet	5862	305	6.03	Blenheim	6181	420	4.65
Cox Orange	13423	1647	6.43	Stark	4376	454	3.88

TABLE III

AVERAGE NUMBER OF SEEDS OF STANDARD VARIETIES WHEN TRIPLOIDS ARE USED AS MALE PARENTS

Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit	Variety	Total blossoms	Total fruit harvested	Average no. seeds per fruit
Gravenstein	8768	61	2.31	Baldwin	9046	783	3.70
King	8643	213	3.60	Spy	10129	262	4.60
Golden Russet	11896	249	3.74	Blenheim	3195	124	3.96
Cox Orange	13125	429	4.29	Stark	3669	186	2.92

Sources of Evidence

The evidence on which the present study is based, comes from two sources, as follows:—

1. The results from breeding work in which a large number of crosses were made, the blossoms being first emasculated.

2. Results obtained from pollination studies, the object of which was simply to test the value of the pollen of different varieties as a means of improving the set of the variety used as a female parent. In these studies emasculation was neglected in order to perform as large a number of pollinations as possible. The limb unit method was used in this work and ten replications of each cross, each on a different tree, were made. The limbs selected were as uniform as could be secured and the trees themselves of uniform age, size and treatment.

All the pollinations were made with pollen previously prepared, and applied to the stigmas as soon as the receptive stage was reached, and, as far as possible, before their own anthers had dehisced. Seed counts from the resulting apples are now available over a four-year period and counts of seedlings resulting from various crosses for two years. In order to make a proper

comparison the two-year figures only are used in the detailed tables. A summary table, showing the four-year results is also given, omitting seedling records.

It is recognized that, from the standpoint of the present paper, possible selfing constitutes a source of error, but it is considered that this does not prevent the results from being significant, since the technique employed tended to reduce selfing to a minimum. Selfing tests conducted under optimum conditions show most of the varieties employed to give remarkably low sets. An exception is Baldwin, and for this reason the figures from all crosses in which Baldwin occurs as a female parent are eliminated from our calculations or shown separately. Furthermore, the results from these tests, with respect to the percentage fruit, seed and seedlings obtained from the various crosses, are consistent with the results obtained from emasculation tests and indicate that the conclusions arrived at on the basis of a study of the data presented in our pollination experiments alone, would be essentially the same as though based on data from experiments in which emasculation was practiced. This seems to show that the final results obtained in the pollination work were not seriously interfered with by selfing. They are therefore presented as being strongly corroborative of those secured in the course of breeding work, in which emasculation was employed throughout.

The data available from the pollination studies are derived from two different sources:—

(a) Results from hand pollination tests with six standard varieties for five years as regards fruit and seed production. In this series fruit and seed data for four years were available, but seedling production for two years only.

(b) Results of various hand pollinations made with Blenheim and Gravenstein as female parents. In these experiments fruit and seed production are also considered, but in this case the seed has been classified, whereas in the other tests the total seed count is used, excluding only the undeveloped seed.

(c) In addition to the foregoing a tabulation has been made to show directly the degree of fruitfulness in relation to seed content.

(d) In order to show the relation between seed content and weight, a correlation between these two factors has been calculated for several standard varieties.

(e) In order to show relation of seed content to premature drop and certain abnormalities of the fruit, data from a series of Gravenstein trees enclosed beneath tents under different conditions of pollination, together with certain other data, are included.

1. *Evidence from Crosses Performed in Breeding Experiments (Series 1)*

These results were obtained in connection with breeding experiments with apple varieties. All trees were enclosed beneath tents and emasculation and hand pollination performed in the usual manner. All blossom clusters were reduced to two blossoms per spur and obviously weak spurs were eliminated. This accounts for the fact that, between Series 1 and Series 2, in the effective

crosses, the former shows a higher percentage success than the latter, in which only the centre bloom was removed and all laterals crossed.

The crosses are classified on the basis of their chromosome constitution, *viz.*, (1) diploid × diploid, (2) diploid × triploid, (3) triploid × diploid and (4) triploid × triploid. In addition, as a matter of interest, results from other crosses involving varieties of unknown constitution, but believed to be either diploids or triploids on the basis of their behavior have been included. It may be stated at this point that the possession of pollen of high germinability and good quality for crossing purposes, with relatively high seed and seedling production, appears to be associated with diploidy and the opposite condition with triploidy.

2. *Evidence from Crosses Obtained in Pollination Tests (Series 2), on Standard Varieties*

These tests were made on non-emasculated blooms, as already described, in the course of pollination experiments with standard varieties of apples. The method of tabulation and terms used are exactly the same as in tables in Series 1. In showing results of triploid × triploid crosses in the detailed tables, figures are given both with and without Baldwin as a female parent. This is because of the high percentage of self-fruitfulness of this variety, which it was considered might affect our results. The figure excluding Baldwin as a female parent is therefore used when discussing general results for crosses of this type. Figures on all points except seedlings are available for the five-year period, 1928-1932 inclusive. Figures including seedlings are available for the years 1930-1931 inclusive. These two sets of figures are given separately to allow for better comparison with the series averages, which only cover the years 1930-1931.

3. *Evidence from Pollination Tests on Gravenstein and Blenheim (Series 3)*

Some evidence may be gleaned from the writers' records of certain crosses made on Blenheim and Gravenstein in 1929. The seed from these two crosses was secured and classified, the percentage fruit and seed obtained from the original blossoms pollinated was calculated, as well as the average number of seeds per fruit for all crosses. Since both these varieties are triploids the results of these crosses are of some interest, since they enable us to study the results in a large number of crosses of both the triploid × triploid and triploid × diploid type, with respect to fruitfulness and the relation of seed production to fruit setting. Unfortunately the chromosome number of all the varieties used as male parents is not known. From their behavior it is believed that most of the varieties of unknown constitution will prove to be diploids.

4. *Evidence Obtained from a Study of the Seed Content of Various Crosses*

In order to bring out more clearly the influence of the pollen parent upon the seed content of the resulting fruit and its relation to fruitfulness, all the fruit obtained from certain standard varieties was classified according to its seed content and the results tabulated to show the degree of fruitfulness obtained with the different seed counts. These data are presented in Table V.

TABLE IV

STANDING OF DIFFERENT TYPES OF CROSSES IN SERIES 1, 2 AND 3

Type of cross	Per cent fruit				Per cent seeds per fruit				Per cent seeds				Per cent seedlings			
	S_1	S_2	S_2a	S_3	S_1	S_2	S_2a	S_3	S_1	S_2	S_2a	S_3	S_1	S_2	S_2a	S_3
Diploid × diploid	23.24	12.11	11.50	—	7.59	7.76	7.09	—	176.4	75.70	77.41	—	115.85	—	38.52	—
Diploid × triploid	0.68	2.89	1.56	—	6.67	4.23	4.04	—	4.50	9.93	6.02		2.70	—	5.17	—
Triploid × diploid	28.46	10.46	9.45	7.41	3.28	4.08	4.41	4.44	93.37	36.79	—		20.86	34.06	8.29	—
Triploid × triploid (excluding Baldwin)	3.15	2.72	2.10	0.41	2.00	3.31	3.39	1.22	6.29	6.81	5.62	—	1.05	—	0.83	—
Triploid × triploid (with Baldwin)			4.71				3.47				13.49				2.91	
Triploid × unknown*	—	—	—	7.23	—	—	—	4.91								
Diploid selfed		1.66				4.36				6.83				2.98		
Triploid selfed		3.56				3.11				9.04				2.07		

S_2, *average for 1927-1932;* S_2a, *average for 1930-1931 only;* **Unknown varieties, probably all diploids;* — *No data.*

TABLE V

TABLE SHOWING PER CENT FRUITFULNESS OF CROSSES IN RELATION TO THE AVERAGE NUMBER OF SEEDS OBTAINED, 1928-1931

Cross	Total blossoms	No. of seeds									
		1–1.9	2–2.9	3–3.9	4–4.9	5–5.9	6–6.9	7–7.9	8–8.9	9–9.9	10–10.9
		Per cent fruitfulness at different seed intervals									
Cox Orange (d) × Baldwin (t)	3719				2.50						
Cox Orange (d) × Golden Russet (d)	4391							14.26			
Cox Orange (d) × Gravenstein (t)	4550				5.41						
Cox Orange (d) × King (t)	5076			2.50							
Cox Orange (d) × McIntosh (d)	3729					10.51					
Cox Orange (d) × Spy (d)	1572							14.44			
Cox Orange (d) × Wagener (d)	3817					14.72					
Cox Orange (d) selfed	6971					1.64					
Golden Russet (d) × Baldwin (t)	3940			2.69							
Golden Russet (d) × Cox Orange (d)	3787					6.07					
Golden Russet (d) × Gravenstein (t)	3853				2.36						
Golden Russet (d) × King (t)	4103			2.56							
Golden Russet (d) × McIntosh (d)	2075						6.84				
Golden Russet (d) selfed	6627			2.02							
Gravenstein (t) × Baldwin (t)	3275		.52								
Gravenstein (t) × Blenheim (t)	821	1.58									
Gravenstein (t) × Cox Orange (d)	4227		15.09								
Gravenstein (t) × Golden Russet (d)	3734			10.39							
Gravenstein (t) × King (t)	4672		1.24								
Gravenstein (t) × McIntosh (d)	3412			9.44							
Gravenstein (t) × Wagener (d)	5586			13.71							
Gravenstein (t) selfed	7908			.87							

TABLE V—*Concluded*

TABLE SHOWING PER CENT FRUITFULNESS OF CROSSES IN RELATION TO THE AVERAGE NUMBER OF SEEDS OBTAINED, 1928-1931

Cross	Total blossoms	No. of seeds									
		1–1.9	2–2.9	3–3.9	4–4.9	5–5.9	6–6.9	7–7.9	8–8.9	9–9.9	10–10.9
		Per cent fruitfulness at different seed intervals									
King (t) × Baldwin (t)	4205			2.81							
King (t) × Blenheim (t)	526				3.04						
King (t) × Cox Orange (d)	4099					4.05					
King (t) × Golden Russet (d)	3792					2.80					
King (t) × Gravenstein (t)	3912			2.89							
King (t) × McIntosh (d)	3137				4.53						
King (t) × Wagener (d)	5104					3.98					
King (t) selfed	6359			2.59							
Spy (d) × Baldwin (t)	3846				2.60						
Spy (d) × Ben Davis (d)	4542								15.59		
Spy (d) × Cox Orange (d)	5011									12.21	
Spy (d) × Golden Russet (d)	5113									9.72	
Spy (d) × Gravenstein (t)	1323					3.85					
Spy (d) × King (t)	4960				3.15						
Spy (d) × Wagener (d)	305										44.92
Spy (d) selfed	6250				1.68						

To permit of more ready comparison of the results obtained from the different types of crosses in the different series the averages for all three series are summarized in Table IV. This will serve to show the general standing of each with respect to fruit, seed and seedlings. The more detailed tables on which this summary is based are placed in an appendix following this article. Attention is particularly called to Tables XIII, XV, XVIII.

In these tables all percentages are based on the original number of blossoms pollinated, which is the reason that percentages of seeds and seedlings may run over 100%. This practice affords a better standard for the desired comparison than any other that could be devised. The average number of seed per fruit is also given, but this figure is not entirely relied upon because some crosses gave a very small number of fruit, sometimes only one, yet the fruit that is obtained may occasionally show a fairly high seed content. When based on such small numbers, however, the result has little or no meaning. The term "per cent fruit" refers to those apples that remain on after the so-called "June drop."

Stayman Winesap has been excluded from the totals in the diploid × diploid class because it does not behave like other varieties of its class, having sticky pollen that agglomerates in bunches and, for this reason, gives poor results in crossing.

Comment on the results as revealed by these tables will be deferred until after the results from other tests are presented.

Discussion of Fruitfulness, Seed Content and Seedling Production

It will be seen from the tables that the general results from Series 1 and Series 2 are strikingly similar in important particulars, although they are based on entirely different varieties. It is apparent at a glance that there is a wide gap dividing the diploid × diploid and the triploid × diploid crosses on the one hand from the diploid × triploid and the triploid × triploid on the other. The former represent a highly fruitful type of cross and the latter a very unfruitful one. Whether diploid × diploid or triploid × diploid crosses come first from the standpoint of fruitfulness depends on the selection of varieties chosen on which to base averages, as there is considerable overlapping in this respect between individual crosses in the two groups. Both diploid and triploid varieties, therefore, are fruitful when crossed by a diploid variety. It is very clearly brought out, however, that, from the standpoint of seed and seedling production, the diploid × diploid crosses exceed the triploid × diploid by a very wide margin, showing that, with the latter type of cross, a high degree of fruitfulness is possible with a lower seed content than is the case with diploid × diploid crosses.

In considering crosses of the unfruitful type, *viz.*, diploid × triploid and triploid × triploid we find that they give poor results on all counts, with some variation between the different series. From the standpoint of percentage fruit, the triploid × triploid type is superior in two out of three

cases and approximately equal in the third case. In several individual cases triploid × triploid crosses were commercially fruitful. In both Series 1 and 2 there have been certain inconsistencies, some giving no fruit and others a fair percentage. Boskoop × Baldwin, for example, gave 7.76% fruit from 116 pollinations, but Boskoop × Blenheim and Boskoop × King gave no fruit at all. Baldwin selfed in Series 2 gave 7.67% fruit with 5046 pollinations, but, in this case, the phenomenon of self-compatibility is involved. Whether the high percentage of fruit obtained by using the pollen of triploid varieties on Baldwin is due to selfing can be determined with certainty only by emasculation tests, but later incomplete investigations indicate that this is not the case. From the breeding standpoint triploid × triploid crosses are mostly worthless, producing in both Series 1 and 2, even fewer seedlings than in the diploid × triploid type. An examination of the detailed figures shows that, in both types there are individual crosses which, though they result in some fruit, produce few fruits with seed and give few or no seedlings, while the seedlings that are produced are of an inferior character or lacking in uniformity. From every point of view, therefore, crosses of these two types are greatly inferior to the other two.

Nevertheless, the comparatively high percentage fruit obtained in certain individual cases of triploid × triploid crosses, which has raised the general average for the group, is one that requires explanation. This is particularly noticeable where Baldwin was used in Series 1 (Table XII, p. 328) but, owing to the possibilities of selfing in Series 2, the results from Baldwin, when used as a female parent, have been excluded. Theoretically, this result might be expected as there is a chance for mutual fertilization of diploid or near diploid gametes in triploid × triploid crosses.

It is noteworthy that all triploid males when used on Baldwin, with the exception of Blenheim, gave a higher percentage of fruit than was obtained by selfing, in spite of the high degree of self-compatibility exhibited by this variety. The possibility, therefore, that this result may be due to the opportunity offered for mutual fertilization of diploid or near diploid gametes should not be excluded.

The inviability of aneuploid types is indicated by the fact that practically all commercial varieties are either diploids or triploids. Though an excess of forms with 41 chromosomes among triploid seedlings is reported by Moffet (17), the only commercial variety recorded as coming in the group is Wellington Bloomless which, according to Nebel (21) has 41 + 1.

It should be borne in mind when considering these results that incompatibility factors have less effect in triploids than in diploids. Furthermore, when comparing triploid × triploid *vs.* triploid × diploid we have to deal with a situation different from that which exists when comparing triploid × triploid with diploid × triploid, since, in unbalanced chromosome types, female gametic fertility is always greater than male.

Considering the evidence gleaned from Series 3 tests and counting only those varieties of known chromosome number, we find, on examining the results from

the triploid × triploid crosses, that (1) in amount of fruit produced from a given number of blossoms, (2) in per cent seed and (3) in average number of seeds per fruit, these crosses fall far below the triploid × diploid crosses. The fact that the triploid × triploid crosses have a small average number of seed and still fewer good seeds, seems to be associated with the unfruitfulness of these crosses. The foregoing results, therefore, strongly corroborate those obtained in the other series. In comparing the results obtained from known diploids with those that may be assumed to be diploids on the basis of their behavior, the similarity is noteworthy.

A study of the data presented in Table V again indicates that seed content must be considered in connection with the chromosome constitution of the varieties concerned. The great difference existing between diploids and triploids as pollinizers is best illustrated in the case of their use on a diploid variety with a comparatively high seed content, like Spy. Here, the fact that all triploid varieties used as male parents gave a low degree of fruitfulness together with a low seed content, and that the reverse held when diploid males were used, is apparent at a glance. The same point may be observed with the other two diploid varieties considered, *viz.*, Golden Russet and Cox Orange. As would be expected, the difference indicated is less apparent when comparatively few-seeded triploid varieties are used as female parents. Nevertheless, even with triploid varieties it will be seen that diploid pollen generally produced a higher seed content, together with a larger number of fruits.

That the degree of fruitfulness is not necessarily in direct proportion to the number of seeds is also apparent. This is particularly true when we compare the few-seeded triploids with the diploids, the former showing a degree of fruitfulness approximately equal to the latter when crossed with a diploid variety, though with only half the seed content. For example, a diploid × diploid cross, Spy × Golden Russet, gave an average seed content of 9.68 and a percentage fruitfulness of 9.72. On the other hand a triploid × diploid cross, Gravenstein × Golden Russet, gave an average seed content of 3.29 and a percentage fruitfulness of 10.39. It will also be noted that, in selfing tests, there is considerable inconsistency between seed content and fruitfulness. Thus, the fruit (1.64%) resulting from selfing Cox Orange blossoms gave a seed content falling in the 5-6 interval, while McIntosh and Wagener, with the same seed content gave 10.51 and 14.72% fruitfulness respectively. Thus it will be seen that, in the fruitful types of cross, whether diploid × diploid or triploid × diploid, we have a higher average seed content than in the unfruitful type, whether triploid × triploid or diploid × triploid.

Attempts to correlate seed content and fruitfulness more closely result in apparent inconsistencies, which might be expected since, in addition to the group factor of a balanced or unbalanced chromosome constitution which we are here especially considering, there are obviously genetic differences, particularly compatibility and incompatibility factors, which enter into the problem.

Relation of Seed Content to Weight

As already indicated, many workers have stated that a correlation exists between weight and seed content in the apple. The fact that one-sided apples show some of the carpels empty on the corresponding side is a matter of general observation. Samples picked at random offer little evidence in this connection, since many factors influence size and weight of fruit, and a disturbing factor is introduced in the utilization of fruits resulting from mixed pollination. On the other hand, trees with a very low set due to an unfruitful cross produce few apples, and those that do set may grow abnormally large owing to favorable nutritional conditions. For this reason, it appears desirable that the samples selected should be produced under uniform and normal conditions. In 1931 two varieties, Gravenstein, as representative of a triploid variety with very low seed content and Northern Spy, representative of a diploid variety with an exceptionally high seed content were selected. A tented tree of each variety which had been provided with a hive of bees and an effective pollinizer, Wagener in the case of Gravenstein, Ben Davis in the case of Spy was used. All the apples on each tree were taken, 500 in the case of Gravenstein and 1,596 in the case of Spy. By thus providing optimum conditions for pollination we naturally reduced the production of abnormal apples likely to result from imperfect fertilization, which undoubtedly affected the results, but gave a value for the effect of seed content.

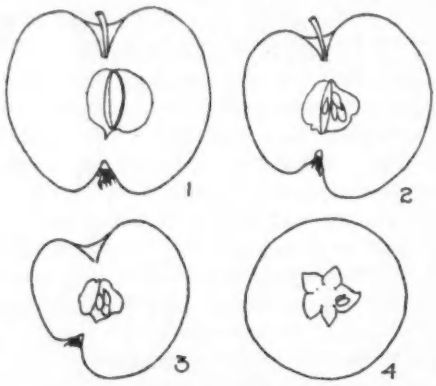
The coefficient of correlation using all seeds, whether filled or not, was, in the case of Gravenstein $.055 \pm .0366$, which is not significant, and for Spy, $.3467 \pm .0148$, which is statistically just significant. Since results with Gravenstein are not in line with those obtained by Einset (6) and since the correlation in the case of Spy is not as great nor as striking as might have been expected, it was decided to duplicate the work with Gravenstein and Spy and, in addition, to run similar correlations with King pollinated with Wagener and Baldwin pollinated with Cox Orange. The following number of fruits were examined: Gravenstein, 1,100; Spy, 1,000; King, 314; and Baldwin, 1,000. The coefficients of correlation obtained in 1932 were as follows: Gravenstein, $.0025 \pm .0302$; King, $.1103 \pm .0557$; Baldwin, $.2723 \pm .0293$; Spy, $.3069 \pm .0286$. In the Gravenstein, King and Baldwin varieties, no significant correlation was obtained, but in the case of the Spy the result may be considered just significant. Since the first three of these are triploids and the last a diploid it was thought that possibly the existence or otherwise of a correlation might depend on chromosome constitution. Accordingly another diploid, *viz.*—Wagener, was selected and one thousand apples from an open pollinated lot used for the purpose of our calculations. A correlation of $.07304 \pm .0315$ was obtained, which is not significant.

The foregoing data show that there is no definite correlation between the weight and the number of seeds per apple in the Gravenstein, King, Wagener and Baldwin varieties under the conditions tested. In the Spy variety a correlation just statistically significant was obtained, but even this cannot be considered at all marked.

Morphological Abnormalities

1. Fruit Distortion

It has already been shown that apples coming off in that wave of abscission to which the name "June drop" is applied have consistently produced a lower average seed content than those that remained on the tree. The relative uniformity in size and weight prevailing among the individual apples on limb-units or even whole trees pollinated with a cross-fruitful variety, has been marked. It has also been noted that, in certain varieties, one-sided apples result from the failure of the seeds on one side to develop. This type of distortion appears to be most pronounced in apples with a relatively high seed content and hence it would be expected that such apples would more commonly occur among the many-seeded diploid variety than among the few-seeded triploid varieties.



Fruit Distortion in Ben Davis

Fig. 1. *Longitudinal section of normal apple containing 15 seeds, viz., 2 carpels with 4 seeds and 2 carpels with 3 seeds.*

Fig. 2. *Longitudinal section of apple with 3 carpels containing 3 seeds each, the remainder empty.*

Fig. 3. *Longitudinal section of apple with 3 seeds in one carpel, the remainder empty.*

Fig. 4. *Cross section of same apple. Note lack of reduction in transverse diameter.*

In such varieties as Spy and Deacon Jones, in which the typical seed content is twenty, distortion of fruit containing less than the normal number of seeds is common. In the case of Spy such fruit is of the characteristic one-sided type. In Deacon Jones a similar distortion is frequently observed and even seedless apples of almost cylindrical shape may be found.

A study of one-sided specimens among Spies revealed an interesting situation. There does not appear to be any reduction in the transverse diameter due to reduced seed content, but there occurs a marked depression of the axial diameter of the fruit on the side where there are no seeds. Out of the one thousand Spies examined 9.30% were thus "drawn" at the calyx-end, giving the one-sided effect. By dividing the apples into two groups, (1) normal and (2) malformed, one hundred in each group, it was found that the average seed content of the first group was 9.74 and of the second 6.05. Further studies on open-pollinated Black Ben Davis gave the results shown in Table VI.

TABLE VI

The relation of seed content to one-sided fruit in Black Ben Davis

Kind of fruit	No. examined	Av. seed content
Normal fruit	103	6.13
Malformed fruit	85	4.54

A similar classification of Ben Davis, also from open-pollinated trees, gave the results shown in Table VII.

TABLE VII

THE RELATION OF SEED CONTENT TO ONE-SIDED FRUIT IN BEN DAVIS

Kind of fruit	No. examined	Av. seed content	Av. no. empty carpels
Normal	94	6.67	0.340
Strongly malformed	79	3.61	2.00
Slightly malformed	30	4.47	1.67

It is unfortunate that conditions did not permit the securing of further data on this point, but it is only to be expected that varieties in which malformed apples are common would show an apparent correlation between seed content and weight, while those that do not respond in this manner to reduced seed content and weight, would show no such correlation. It would also be expected that more distortion would occur under conditions of insufficient pollination than would be the case where conditions for adequate pollination were provided, as with the tented trees. Hence, by selecting only fruit from well-pollinated trees, we secure lower values than would otherwise be the case.

Among varieties in which one-sided or distorted apples were not found in these studies were Gravenstein, King, Baldwin, Wagener and Blenheim. It is of interest to note that in the first four of these the writers were unable to demonstrate any significant correlation between seed content and weight. Regarding the fifth variety they have no information on this point.

2. PREMATURE DROP AND OPEN CALYX-END

(a) Preliminary Studies

In one of the varieties mentioned, *viz.*, Gravenstein, in which no correlation between seed content and weight was demonstrated and in which no distortion of the fruit due to failure of seeds to develop has been found, data bearing on the influence of imperfect pollination and consequent low seed content are available. It should be noted that, in this variety, the average seed content is lowest of all varieties studied. In addition, the phenomenon of self-compatibility is marked, comparing with Spy in this respect. It has long been noted in Gravenstein that there occurs, in certain seasons, a later drop of practically fully grown apples that ripen prematurely and drop in late August and early September. This condition was very pronounced in the season of 1928 and it was noted that such apples also had a lower seed content than apples remaining on the tree. In 1932 more definite evidence was obtained that appeared to connect premature drop with imperfect pollination and low seed content. This evidence was obtained from a series of Gravenstein trees tented during bloom, some of which were (1) supplied with bees to serve as pollinators, and with a source of foreign pollen in the form of a "bouquet" of blossoming limbs of the desired variety, (2) without bees, and (3) with neither bees nor bouquets, as indicated later. The results were compared

with those from an open-pollinated untented tree. While this tree received a measure of effective pollination, it is only reasonable to suppose that the pollination was not as complete as with tented trees. Though based only on a single season's observations, the results appear sufficiently suggestive to justify consideration at this point. The need of much further work in order definitely to settle the different issues raised is clearly indicated.

The first fact noted in connection with these tests is that a comparatively large number of drops were present under the open-pollinated tree, whereas the Wagener-pollinated tree showed very few drops. So little fruit was obtained from the selfed or ineffectively pollinated trees, that the results from such trees were not significant.

Examination of the drop fruit from the two trees referred to showed that not only were the drop apples less numerous beneath the Wagener-pollinated tree, but that the content of developed seeds was greater and that of undeveloped seeds, represented by fragments of undeveloped embryos, less than those beneath the open pollinated tree.

The detailed results of the examination of these drops are shown in Table VIII.

TABLE VIII

THE RELATION OF SEED CONTENT TO PREMATURE DROP OF GRAVENSTEIN

Treatment	Number drops	Av. no. seeds	Av. no. undeveloped seeds
Bees and Wagener bouquets	134	5.24	2.28
Open-pollinated	289	3.67	3.29

On cutting open the apples to make the seed count it was noted that the structure of many of the drop apples was abnormal, in that the calyx-end was not closed in the ordinary way. The cavity, sometimes erroneously called the "calyx tube" in such apples, extended downward penetrating the core lines and resulted in an open-core condition. This open-core condition had evidently exposed the core region to the invasion of certain saprophytic organisms resulting in the condition known as "moldy core." A striking difference between the incidence of moldy core in the two series was at once apparent, *viz.*, 5.22% from the Wagener-pollinated and 35.29% from the open-pollinated. An accurate count showing the percentage of open blossom end present in the two sets of drops is not available.

In view of the foregoing results it was decided to examine the apples that still remained on the trees throughout the entire series in order to determine: (1) whether a definite relation existed between seed content and the open-core condition on an open-pollinated tree, where a measure of effective pollination had been obtained, and (2) to study the effect of different degrees of effectiveness of pollination on the same condition.

(b) Procedure and Results

In connection with the first study individual apples were cut from an open-pollinated tree and classified as to seed content and open or closed calyx.

For the second study fruit from each of the six tented trees was saved and similarly classified. In the case of the trees that yielded small crops due to lack of suitable pollinizers or absence of pollinators, the entire crop was harvested and examined. From the remaining trees (*i.e.*, the open-pollinated and that supplied with bees and Wagener bouquets) sufficient apples were removed to ensure a satisfactory average.

TABLE IX

THE RELATION BETWEEN MOLDY CORE AND OPEN CALYX-END

Condition	No. fruit	Av. seeds	% fruit
Non-moldy and closed	997	3.54	83.08
Moldy and open	187	3.24	15.58
Moldy and closed	13	3.08	1.08
Non-moldy and open	3	3.00	0.25
	1200		

The results from an examination of 1200 apples taken from the open-pollinated tree are classified in the accompanying table with respect to total number and percentage of fruits affected with "moldy core," together with the average seed content for the different groups.

The foregoing results show clearly that the open blossom-end condition is associated with "moldy core." The difference in the average seed content between fruits with open and closed calyx-end does not seem great, but it evidently is great enough to be highly significant as indicated in the results from an examination of the fruit from the entire tented series. This is indicated in the next table.

TABLE X

SEED CONTENT, OPEN CALYX-END AND MOLDY CORE UNDER DIFFERENT CONDITIONS OF CONTROLLED POLLINATION

Treatment	No. fruit	Av. seeds per apple	% seeds	Av. undeveloped seeds per apple*	% calyx open	% moldy core
No bees and no bouquets	28	1.14	47.06	1.29	57.14	71.43
Bees and no bouquets	194	1.46	22.29	5.10	45.88	57.73
Bees and Blenheim (triploid) bouquets	116	1.27	16.84	6.26	37.93	37.07
No bees and Wagener (diploid) bouquets**	435	2.30	28.71	5.71	26.90	24.14
Open pollinated	1200	3.49	50.11	3.47	15.83	16.67
Bees and Wagener (diploid) bouquets	1100	4.73	62.89	2.79	0.82	1.00

**Undeveloped seeds were those that were visible as very minute brown specks, representing seeds that had not developed in normal manner.*

***Pollination was effected by a blast of air from an orchard duster blowing through the bouquets and over the tree.*

(c) Discussion

The association between the degree of pollination, seed content, the amount of open calyx-end and of moldy core appears evident from a study of these figures. To emphasize the close relation between seed content and open

calyx-end, the fruit has again been classified according to seed content, regardless of its origin, and the results presented in the next table. Four intervals are given, *viz.*, (1) over one but less than two, (2) over two but less than three, (3) over three but less than four, and (4) over four.

TABLE XI

RELATIONSHIP OF AVERAGE SEED CONTENT TO OPEN CALYX

Good seeds	Av. no. seeds	Av. no. of undeveloped seeds	No. of fruit examined	% with open calyx-end
Over 1	1.38	5.18	338	44.08
Over 2	2.30	5.71	435	26.90
Over 3	3.49	3.47	1200	15.83
Over 4	4.73	2.79	1100	.82

The conclusion that an increasing seed content is associated with a decreasing percentage of fruit with an open calyx-end would appear justified from the results. There is also a correlation between the number of undeveloped or aborted seeds and closure of the calyx-end, but, in this case a large number of aborted seeds is associated with a high percentage of apples with the calyx-end open and *vice versa*. In the average number of undeveloped seeds there is one apparent contradiction. The number is larger in the group "over 2 seeds" than in the group, "over 1 seed." This may be explained by referring to Table XI. Where no bees or no bouquets were used, in addition to obtaining few seeds in the apples, very few aborted seeds were produced. This materially lowers the average number of aborted seeds for this group.

The effect of the degree of pollination that takes place is apparent from these figures. Thus, where bees and an effective pollinizer, namely, Wagener, were used, 4.23 seeds per apple were produced and only .82% of the fruits had an open calyx-end. When an ineffective pollinizer, Blenheim, was used, the number of seeds per apple was reduced to 1.27 and the percentage of fruit with open calyx-end increased to 37.93%. The results from the open pollinated tree, which was, however, surrounded with both effective and ineffective pollinizers, were intermediate.

These results, based on one year's figures, very clearly indicate that there is a definite relation between the effectiveness of pollination and the closure of the calyx-end. The fact that this "calyx tube" does not close exposes the seed cavity to infection from various organisms, and this would appear to be the true cause of moldy core, the associated organisms being secondary.

The fact that the condition was particularly severe in 1932, when weather conditions during blossoming were unusually unfavorable for pollination, is of interest. No attempt was made to make any further studies of moldy core, nor of the organisms associated therewith. Nor has a study of the development of the apples resulting in open or closed calyx-end been at-

tempted. This would require a complete study of the development of the fruit from the time of fertilization onward. However, a study of the calyx-end or "eye" of the apple has been recently made by Tetley (22) in connection with disease known as "eye-rot" caused by *Nectria galligena*. She notes that the tube formed by the style may, in the Bramley seedlings, be open all the way down into the core exactly as has been noted in the case of Gravenstein. Usually, however, the way is blocked owing to the interlocking and growing together of some of the surface cells. It is pointed out that in very young apples before they have set, the calyx cavity is shallow and open, since the sepals project horizontally from the apex of the apple. When the apple has set, the rapid growth of the fruit brings the base of the sepals into a horizontal position, thus closing the cavity to a very large extent.

No extensive survey has been made to detern ine the occurrence of this condition in apple varieties, but in addition to Gravenstein and Bramley seedling, we have noted it also in another triploid variety, *viz.*, Boskoop. This is of interest in view of the observed fact that, in triploids, abnormalities are likely to develop late in the life cycle. The evidence presented above is not sufficient to permit of too definite conclusions, nor is it possible to state that no other factors, besides those indicated, have a bearing on the problem.

Summary

1. Results obtained from apple crosses in which the limb unit method was employed and a large number of pollinations performed without emasculation, gave sufficiently comparable results to those obtained with fewer numbers with emasculation, to afford useful corroborative evidence as to the behavior of the different types of crosses.

2. Large counts of seed content from drop and picked apples show a consistently larger average number in the latter, indicating the importance of seed content in fruit setting.

3. From the standpoint of fruitfulness, both diploids and triploids as female parents are fruitful when crossed with diploid varieties.

4. Both diploids and triploids, as female parents, are inferior in fruitfulness when crossed with triploid varieties, considerable overlapping again occurring between individual crosses, particularly in the triploid × triploid group, some of which were fairly fruitful. The diploid × triploid cross is uniformly unfruitful.

5. Self-compatibility is a varietal characteristic. The relatively high self-compatibility of Baldwin, as evidenced by percentage fruit, seed and seedlings produced, is particularly noticeable.

6. Diploid varieties, as female parents, have consistently given a higher seed content than triploid varieties and, within the variety, the seed content is affected by the chromosome constitution of the male parent, diploids as male parents giving a higher seed content than triploids.

7. Though diploid × diploid and triploid × diploid crosses are on the average of approximately equal fruitfulness, the latter have a much lower average seed content. The percentage fruitfulness is not necessarily directly proportional to the seed content, as is explained more fully elsewhere.

8. Though triploid × triploid crosses may sometimes exceed the diploid × diploid type in fruitfulness, the latter have a higher average seed content.

9. As might be expected, the results obtained from seedlings are in general agreement with those based on seed alone, and the order of seedling production is the same in all tests, as follows: first, diploid × diploid; second, triploid × diploid; third, diploid × triploid; and fourth, triploid × triploid.

10. Data not included in the foregoing paper indicate that, whereas the male parent has no measurable effect on germination, triploids as a group show a much lower germination than diploids when used as females.

11. Tests conducted in 1931 showed no correlation between seed content and weight in the case of Gravenstein, and a barely significant correlation in the case of Spy, results which were confirmed in 1932. Further tests conducted with Wagener, Baldwin and King in 1932 gave no significant correlation with those varieties.

12. In apple varieties with a normally high seed content, failure of seed to develop on one side of an apple results in distorted or "one-sided" fruit. In certain varieties also, seedless or nearly seedless apples have been found in which the distortion extends to both sides and the fruit is almost cylindrical in shape.

13. Examination of "one-sided" and normal apples from open pollinated trees revealed the fact that, in all cases, the average number of seeds was lower and the average number of empty carpels was higher in the former group.

14. Imperfect pollination, low seed content, open calyx-end and "moldy core," appear, on the basis of one season's observations, to be associated phenomena in Gravensteins, but further work is necessary before definite conclusions are possible.

Appendix

The following are the detailed tables upon which the foregoing discussion has been largely based. Attention is particularly called to the Summary Tables XIII, XV, and the last three lines of Table XVIII.

FIG. 1. *General view of seedlings.*

FIG. 2. *29. Cox Orange × G. Russet (Diploid × diploid). 33. King × G. Russet (Triploid × diploid). 34. Spy × Cox Orange (Diploid × diploid).*

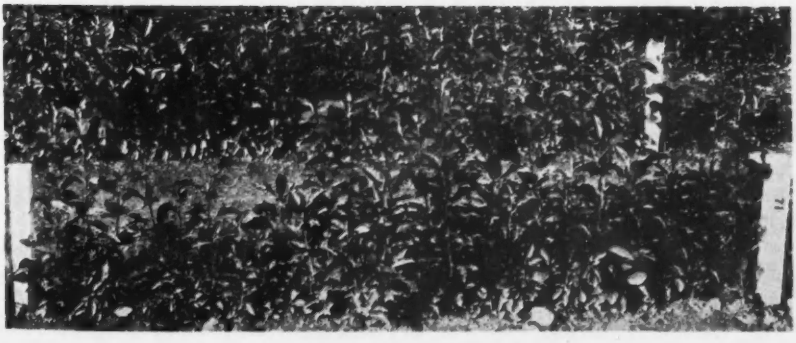


FIG. 3. *71. Cox Orange (Diploid) selfed*

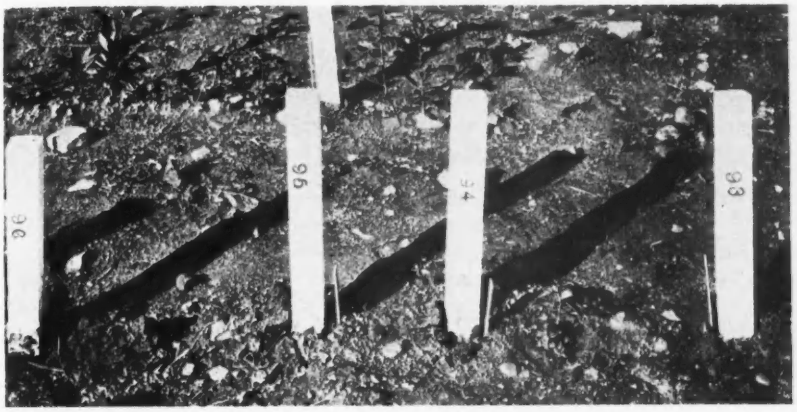


FIG. 4. *93. Russet × Gravenstein (Diploid × triploid). 94. Blenheim × Gravenstein (Triploid × triploid). 95. McIntosh × Gravenstein (Diploid × triploid). 96. Baldwin × Gravenstein (Triploid × triploid).*

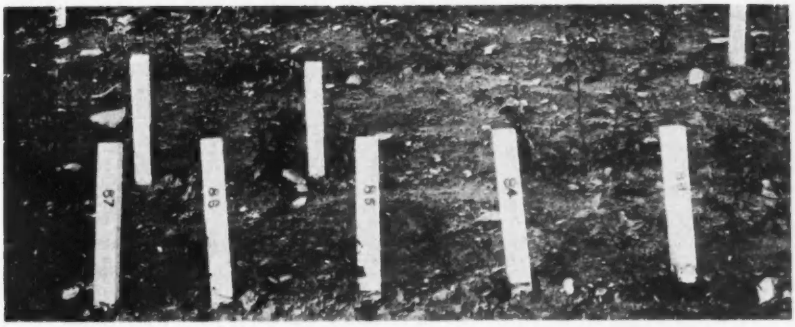


FIG. 5. *83. Stark × Fallawater (Triploid × triploid). 84. Blenheim × Duchess (Triploid × ?). 85. Blenheim × Wagener (Triploid × diploid). 86. Stark × Fameuse (Triploid × diploid). 87. Blenheim × Fallawater (Triploid × triploid).*

TABLE XII

RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: diploid × diploid					
Delicious × Ben Davis	48	37.50	289.58	7.72	185.42
Delicious × Cox Orange	57	24.56	154.39	6.27	122.81
Delicious × Duchess of Oldenberg	53	13.21	107.77	7.85	81.13
Delicious × Golden Russet	64	21.87	184.38	8.43	142.19
Delicious × McIntosh	46	15.22	108.69	7.14	71.74
Delicious × Rome Beauty	43	11.63	97.67	8.40	5.17
Delicious × Northern Spy	61	22.95	157.38	6.86	122.95
Delicious × Stayman Winesap	47	—	—	—	—
Ben Davis × Delicious	28	14.28	114.29	8.00	92.86
Rome Beauty × Delicious	16	31.25	112.50	3.60	75.00
Cox Orange × Delicious	22	27.27	219.18	8.00	161.54
McIntosh × Delicious	8	25.00	137.50	5.50	12.50
Red Rome Beauty × Cox Orange	4	25.00	200.00	8.00	175.00
Red Rome Beauty × S. Winesap	7	—	—	—	0.00
Red Spy × Red Rome Beauty	49	44.90	387.75	8.59	198.78
Red Spy × Cox Orange	41	21.95	197.56	9.00	102.44
Red Spy × McIntosh	28	14.25	96.43	6.75	21.43
Red Spy × Stayman Winesap	10	—	—	—	0.00
Type of cross: diploid × triploid					
Delicious × Baldwin	71	1.41	8.45	6.00	8.45
Delicious × Crimson Bramley	39	—	—	—	—
Delicious × Gravenstein	59	3.42	23.73	7.00	8.47
Delicious × King	81	—	—	—	—
Delicious × Ribston	50	—	—	—	—
Delicious × Stark*	68	—	—	—	—
Delicious × Nonpareil*	74	6.75	42.02	6.20	28.38
Deacon Jones × Gravenstein	42	—	—	—	—
Deacon Jones × Crimson Bramley	33	—	—	—	—
Deacon Jones × Baldwin	28	—	—	—	—
Deacon Jones × Ribston	19	—	—	—	—
Deacon Jones × Blenheim	22	—	—	—	—
Deacon Jones × Stark*	28	3.57	7.14	2.00	3.57

**Assumed to be triploids on account of their genetic behavior; later determined as such by Dr. M. V. Roscoe.*

TABLE XII—*Continued*

RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: triploid × diploid					
Boskoop × Red Astrachan	96	12.50	38.54	3.08	15.63
Boskoop × Ben Davis	89	29.21	84.26	2.88	1.12
Boskoop × Cox Orange	61	24.59	98.36	4.00	39.34
Boskoop × Delicious	122	28.70	77.87	2.71	16.39
Boskoop × Duchess of Oldenberg	104	40.38	149.03	3.69	40.38
Boskoop × Golden Russet	112	39.29	132.14	3.36	28.57
Boskoop × McIntosh	89	13.48	30.34	2.25	5.62
Boskoop × Red Rome Beauty	102	27.55	96.08	3.50	14.71
Boskoop × Northern Spy	118	35.60	119.49	3.35	25.42
Boskoop × Stayman Winesap	97	20.60	59.79	2.90	10.31
Ribston × Delicious	16	31.25	112.50	3.60	12.50
Baldwin × Delicious	11	63.63	390.90	6.14	63.64
Crimson Bramley × Delicious	9	44.44	33.33	.75	11.11
Type of cross: triploid × triploid					
Boskoop × Baldwin	116	7.76	15.52	2.00	2.59
Boskoop × Blenheim	78	—	—	—	—
Boskoop × King	92	—	—	—	—
Boskoop × Stark	103	1.94	4.85	2.50	0.00
Boskoop selfed	62	3.22	11.29	3.50	0.00
Type of cross: triploid × assumed diploids					
Boskoop × Adams Pearmain	118	21.18	106.70	5.04	22.88
Boskoop × Deacon Jones*	143	15.38	58.04	3.27	15.38
Boskoop × Grimes Golden*	96	38.54	123.95	3.21	33.33
Boskoop × Opalescent	112	33.03	111.61	3.38	45.75
Boskoop × Yellow Transparent	113	17.70	51.33	2.90	19.47
Type of cross: diploid × assumed diploids					
Delicious × A. Pearmain	62	6.45	40.32	6.25	29.03
Delicious × Deacon Jones†	86	38.37	261.62	6.82	194.19
Delicious × Golden Russet†	67	23.38	106.53	7.27	114.93
Delicious × Jonathan†	58	15.52	110.34	7.11	75.86
Delicious × Yellow Transparent	56	50.00	303.57	7.39	205.36
Ben Davis × Deacon Jones†	36	16.66	88.82	5.33	63.89
Rome Beauty × Deacon Jones†	13	69.23	146.15	2.11	76.92
R. Rome Beauty × Deacon Jones†	7	28.57	200.00	7.00	128.57
R. Rome Beauty × York Imperial	4	75.00	700.00	9.33	200.00
Red Spy × Deacon Jones†	15	13.33	140.00	10.50	73.33
Red Spy × Jonathan†	22	27.27	195.45	7.16	104.55
Red Spy × York Imperial	20	25.00	190.00	7.60	115.00

**Since writing the foregoing the chromosome number of these varieties has been determined as 34 by Dr. M. V. Roscoe.*

†*Since writing the foregoing these varieties have been shown to be diploids by Dr. M. V. Roscoe.*

TABLE XII—*Continued*

RESULTS OF VARIOUS APPLE CROSSES, 1930-1931 (Series 1)

Cross	Total blossoms	% fruit	% seeds	Av. no. seeds per fruit	% viable seedlings 1928-1931
Type of cross: assumed diploid × diploid					
Deacon Jones × Red Astrachan	47	19.05	129.78	6.77	78.72
Deacon Jones × Ben Davis	28	21.43	128.57	6.00	67.86
Deacon Jones × Delicious	45	28.89	224.44	7.77	117.78
Deacon Jones × Duchess of Oldenberg	28	10.71	78.57	7.33	53.57
Deacon Jones × McIntosh	26	5.27	5.27	1.00	5.27
Deacon Jones × Yellow Bellflower	22	13.64	100.00	7.33	63.64
Deacon Jones × Stayman Winesap	29	3.45	3.48	1.00	0.00

TABLE XIII

SUMMARY OF RESULTS FROM DIFFERENT TYPES OF CROSSES, 1930-1931 (Series 1)

Diploid × diploid	568	23.24	176.41	7.59	115.85
Diploid × triploid	444	0.68	4.50	6.67	2.70
Triploid × diploid	1026	28.46	93.37	3.28	20.86
Triploid × triploid	286	3.15	6.29	2.00	1.05
Assumed diploid × diploid	225	16.00	108.44	6.78	61.72
Diploid × diploid (without Stayman Winesap)	196	17.86	123.98	6.94	70.92
Diploid × assumed diploid	446	26.23	176.68	6.74	118.39
Diploid × triploid (with Stark and Nonpareil)	614	1.47	9.28	6.33	5.37
Triploid × assumed diploid	582	24.26	87.80	3.62	26.12
Triploid × triploid (with Stark)	389	2.83	5.91	2.09	0.77
Triploid selfed	62	3.22	11.29	3.50	—
Diploid selfed	67	1.49	11.94	8.00	2.99

TABLE XIV

PER CENT FRUIT, SEEDS, AND SEEDLINGS PRODUCED FROM VARIOUS APPLE CROSSES, SERIES 2, 1930-1931

Cross	Number blossoms pollinated	Av. % fruit after July drop	Av. no. seeds per fruit	% seeds 1930-31	Av. % seedlings 1930 and 1931
Diploid × diploid					
Cox Orange × Golden Russet	3207	15.47	6.79	100.31	49.83
Cox Orange × McIntosh	3298	9.43	6.47	57.10	29.78
Cox Orange × Wagener	3258	13.35	6.23	75.38	28.97
Cox Orange selfed	5018	1.79	5.22	9.05	3.51
Spy × Ben Davis	4542	15.59	8.70	114.88	63.41
Spy × Cox Orange	3674	10.23	8.86	76.24	49.80
Spy × Golden Russet	3551	10.59	9.61	88.26	48.27
Spy selfed	4058	1.89	4.66	6.09	3.95
Golden Russet × Cox Orange	2602	6.53	6.54	32.67	17.10
Golden Russet × McIntosh	2075	6.84	6.27	35.04	13.78
Golden Russet selfed	3892	0.90	2.86	2.06	1.28

TABLE XIV—*Continued*

PER CENT FRUIT, SEEDS, AND SEEDLINGS PRODUCED FROM VARIOUS APPLE CROSSES, SERIES 2, 1930-1931

Cross	Number blossoms pollinated	Av. % fruit after July drop	Av. no. seeds per fruit	% seeds 1930-31	Av. % seedlings 1930 and 1931
Diploid × triploid					
Cox Orange × Baldwin	2420	3.24	4.68	13.35	5.21
Cox Orange × Gravenstein	3283	5.88	4.84	25.84	13.80
Cox Orange × King	3177	1.92	3.85	6.55	3.02
Spy × Baldwin	2649	2.15	4.38	7.93	5.02
Spy × King	3227	1.95	3.76	5.83	3.35
Golden Russet × Gravenstein	2379	2.69	4.13	8.15	3.54
Golden Russet × King	2581	2.63	3.61	6.86	2.36
Golden Russet × Baldwin	2465	2.31	3.33	7.01	3.49
Triploid × diploid					
Gravenstein × Cox Orange	2776	16.57	2.93	42.36	9.04
Gravenstein × Golden Russet	2753	11.12	3.00	26.63	4.32
Gravenstein × McIntosh	2064	13.08	3.35	29.36	5.72
Gravenstein × Wagener	4175	15.38	3.66	46.11	14.35
King × Cox Orange	2967	2.83*	5.46	13.25	2.26
King × Golden Russet	2702	3.00	5.08	12.40	4.15
King × McIntosh	2755	4.21	4.19	15.06	3.27
King × Wagener	3496	3.15	5.39	15.10	1.43
Baldwin × Cox Orange	3949	11.47	4.93	48.67	17.30
Baldwin × Golden Russet	3307	9.13	4.31	35.56	7.14
Baldwin × Spy	3575	9.87	5.38	46.24	12.67
Triploid × triploid					
Gravenstein × Baldwin	2451	0.57	2.80	1.14	0.20
Gravenstein × King	3159	1.08	2.58	1.96	0.32
Gravenstein × Blenheim	162	3.70	1.60	4.94	1.23
Gravenstein selfed	5581	1.00	3.38	2.85	0.20
King × Baldwin	3008	3.59	3.19	9.54	1.50
King × Gravenstein	2621	3.40	3.75	10.87	1.41
King × Blenheim	134	0.00	0.00	0.00	0.00
King selfed	5106	2.82	2.88	6.54	0.74
Baldwin × Gravenstein	3227	9.30	3.53	30.77	7.91
Baldwin × King	3066	7.66	3.41	24.69	5.97
Baldwin × Nonpareil	139	8.63	2.09	16.55	4.32
Baldwin × R. I. Greening	458	6.33	2.96	14.85	1.09
Baldwin selfed	5046	7.67	3.19	20.21	5.47

**This is lower than the average figure which is 5.08% for the period 1928-1932 inclusive.*

TABLE XV

SUMMARY OF RESULTS FROM DIFFERENT TYPES OF CROSSES, 1928-1932 (Series 2)

Type of cross	Total blossoms	% fruit	% seeds*	Av. no. seeds per fruit*	% viable seedlings 1930-31
Diploid × diploid	43,294	12.11	75.70	7.07	38.52
Diploid × triploid	46,426	2.89	9.93	4.04	5.17
Triploid × diploid	60,284	10.46	36.79	4.41	8.29
Triploid × triploid (excluding Baldwin as female parent)	21,645	2.72	6.81	3.39	0.83
Triploid × triploid	32,267	4.71	13.49	3.47	2.91
Diploid selfed	22,894	1.66	6.38	4.36	2.98
Triploid selfed	22,021	3.56	9.04	3.11	2.07

**Seed counts made on basis of actual number of fruit harvested.*

TABLE XVI

TABLE SHOWING STANDING OF DIFFERENT TYPES OF CROSSES IN SERIES 2 FOR FIVE YEARS

Type of cross	% fruit	Av. seeds per fruit	% seeds	Type of cross	% fruit	Av. seeds per fruit	% seeds
Diploid × diploid	12.11	7.07	75.70	Triploid × diploid	10.46	4.41	36.79
Diploid × triploid	2.89	4.04	9.93	Triploid (excluding Baldwin) × triploid	2.72	3.39	6.81

TABLE XVII

TABLE SHOWING STANDING OF DIFFERENT TYPES OF CROSSES FOR SERIES 1 AND 2 FOR TWO YEARS

Type of cross	% fruit		Av. seeds per fruit		% seeds		% seedlings	
	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2	Sereis 1	Series 2
Diploid × diploid	23.24	11.50	7.59	7.60	176.41	77.41	115.85	38.52
Diploid × triploid	0.68	1.56	6.67	4.20	4.50	6.02	2.70	5.17
Triploid × diploid	28.46	9.45	3.28	4.08	93.37	10.51	20.86	8.29
Triploid × triploid (excluding Baldwin)	3.15	2.10	2.00	3.27	6.29	5.62	1.05	0.83

TABLE XVIII

RESULTS IN FRUIT AND SEED PRODUCTION OF CERTAIN CROSSES WITH BLENHEIM AND GRAVENSTEIN, 1929 (Series 3)

Cross	Number blossoms pollinated	Total no. of fruit	Total no. of seeds				Av. no. seeds per fruit	Av. good seeds per fruit	% good seed	% fruit
			Good seeds	Poor seeds	Total seeds	Unde-veloped				
Blenheim × Delicious (?)	73	10	18	40	58	31	5.80	1.80	24.66	15.07
Blenheim × Duchess (?)	148	20	54	29	83	85	4.15	2.70	36.49	13.51
Blenheim × Jonathan (A)	160	8	33	12	45	22	5.63	4.13	20.63	6.88
Blenheim × McIntosh (d)	90	13	27	31	58	27	4.46	2.08	30.00	16.67
Blenheim × Nonpareil (t) (Roxbury Russet)	199	1	3	0	3	5	3.00	3.00	1.51	0.52
Blenheim × Red Astrachan (d)	203	21	59	41	100	73	4.76	2.81	29.06	10.84
Blenheim × Ribston (t)	107	0	0	0	0	0	0	0	0	0
Blenheim × Rome Beauty (d)	127	13	50	19	69	43	5.31	3.85	39.37	10.24
Blenheim × Wagener (A)	172	15	69	30	99	29	6.60	4.60	40.12	9.30
Blenheim × Wealthy (d)	181	18	45	32	77	61	4.28	2.50	24.86	10.50
Blenheim × Wolf River (A)	149	18	56	62	118	55	6.56	3.11	37.58	14.77
Blenheim × Yellow Transparent (X)	204	15	54	36	90	35	6.00	3.60	26.47	7.35
Blenheim × York Imperial (?)	112	14	56	29	85	29	6.07	4.00	50.00	13.39
Gravenstein × Baldwin (t)	824	3	4	3	7	25	2.67	1.33	0.49	0.36
Gravenstein × Blenheim (t)	—	4	7	2	9	40	2.25	1.75	No record	
Gravenstein × Cox Orange (d)	1040	56	133	95	228	328	4.07	2.38	12.79	6.35
Gravenstein × Duchess (?)	428	16	27	43	70	102	4.38	1.69	6.31	3.97
Gravenstein × Golden Russet (d)	981	75	179	137	316	507	4.21	2.39	18.25	8.36
Gravenstein × Gravenstein (t)	2148	11	10	16	26	106	2.45	0.91	0.47	0.61
Gravenstein × King (t)	1079	3	3	1	4	35	1.33	1.00	0.28	0.28
Gravenstein × McIntosh (d)	340	42	66	122	188	250	4.48	1.57	19.41	4.67
Gravenstein × Red Astrachan (d)	398	23	60	58	118	144	5.13	2.61	15.08	5.78
Gravenstein × Spy (d)	288	10	28	22	50	59	5.00	2.80	9.72	3.82
Gravenstein × Stark (t)	1098	52	142	151	293	279	5.63	2.73	12.93	5.19
Gravenstein × Wagener (X)	745	120	375	243	618	611	5.15	3.13	50.34	6.04
Gravenstein × Wolf River (X)	843	39	95	99	194	219	4.97	2.44	11.27	5.58
Gravenstein × Yellow Transparent (?)	1158	49	128	135	263	279	5.37	2.61	11.05	4.66
Triploid × known triploid	4357	18	20	20	40	171	1.22	0.61	0.46	0.41
Triploid × all others (X)	8948	647	1754	1425	3179	3268	4.91	2.71	19.60	7.23
Triploid × known diploid	3658	271	647	557	1204	1492	4.44	2.39	17.69	7.41

(t) *represents triploids;* (d) *diploids;* (?) *unknown;* X, *classified as unknown at time of writing, but since shown to be diploids;* A, *probably diploid. Crosses with known diploids are given for comparison.*

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A STUDY IN THE RELATIVE CONSTANCY OF HIVE BEES AND WILD BEES IN POLLEN GATHERING[1]

By W. H. Brittain[2] and Dorothy E. Newton[3]

Abstract

The chief purpose of the investigations described in this paper was to determine the relative pollen constancy of the various insect pollinators of the apple. In addition to the hive bee, these proved to be solitary bees belonging to the genera *Halictus* and *Andrena*, with Bremidae and various Diptera playing a minor role. It is pointed out that apparent flower constancy depends a great deal upon availability and that almost any result may be obtained by choosing certain periods in which to make tests. The results, based on analyses of the pollen loads of bees captured in apple blossoms, place the hive bee first as regards the number of pure loads, followed by *Halictus*, with *Andrena* a poor third. Taking into consideration the results of the entire season, and the analyses of bees from different sources of pollen, *Halictus* came first in these particular tests, but the difference is not considered significant. The supposed superiority of the hive bee from the standpoint of constancy does not appear to have been proved. Both *Halictus* and the hive bee, however, evidenced a significant superiority over the *Andrena* species studied.

Review of the Literature

Aristotle is authority for the statement that, "On each trip the bee does not fly from the flower of one species to that of another, but goes, for example, from violet to violet, without touching any other flower before returning to the hive." Darwin (10) also believed that bees exercised discrimination in their visits, the nectar gatherers choosing bloom that gives the most nectar in the easiest way and the pollen gatherers similarly, in the case of pollen. He considers both hive and bumble bees to be good botanists, recognizing varieties of the same species even though of different color. He does not consider the habit of constancy to be invariable, however, especially when only a few plants of the same species grow together. He emphasizes the importance to the plant of the habit of constancy in helping to ensure cross-pollination.

Since Darwin's time a great deal of evidence, much of it conflicting, has been brought forward regarding constancy in bees. Among these contributions may be mentioned those of Bennett (1, 2, 3, 4), Christy (9), Mueller (15), Bulman (7), Ord (16), Plateau (20), Pérez (18), Wagner (23), Lovell (14), Robertson (21), and Kranichfeld (13). Most of these papers and others not mentioned have been carefully reviewed by Clements and Long (8) and need not be repeated. The work of Bonnier (6) on the division of labor among bees, however, deserves mention. This worker, by a system of marking and subsequent observations of the marked bees, claims to have demon-

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Part of this investigation was carried on at Macdonald College, Quebec, through funds supplied by that institution, and the remainder at various points in Kings County, Nova Scotia, as a part of the project in apple pollination carried out under the direction of a committee of the Dominion Department of Agriculture, of which Mr. Arthur Gibson, Dominion Entomologist, was chairman.

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strated that workers are divided into scouts and collectors, which duties however, may be interchanged at different periods. The collectors he divides into nectar collectors, pollen collectors and propolis collectors, and states that each group of collectors is, for the time being, faithful to its particular duty. Clements and Long (8) report that, in their own studies, "The analysis of pollen loads showed that 86 individuals carried mixed loads in comparison with 121 carrying pure pollen. With respect to species, none represented by five or more individuals was characterized by pure loads, the latter being found only on four bees belonging to *Halictus pulzenus*, on three of *Monumetha albifrons*, and two of *Osmia melanotricha*. In *Apis* the relation of pure loads to mixed was 28 : 3, in *Andrena crataegi* 15 : 3, in *Bombus juxtus* 19 : 6, and in *Bifarius* 7 : 5. Variation in behavior within a genus is shown by the fact that for *B. occidentalis* the ratio was 9 : 17, *Andrena madronitens* 1 : 3, and *A. vicina* 2 : 3, while for *Halictus medionitens* it was 2 : 2. It is clear that bees in general are no more constant in the collection of pollen than in the gathering of nectar."

The question of whether bees remain constant to the same flower throughout its blooming period or whether the constancy applies only to individual bees is left unsettled by most of the earlier workers, or the two types of constancy are not clearly distinguished. In this connection some more recent observations may be quoted. Parker (17) observed hive bees at work on a mixed patch of boneset and aster; they confined themselves at one period to the boneset, at another to aster. Occasionally they alighted accidentally on the flowers which they were not working at the time, but immediately left them. The same behavior was noted in mixtures of *Spiraea* and *Lonicera;* of *Philadelphus* and *Physocarpus;* and of *Taraxacum* and *Melilotus*. However, it was also observed that bees occasionally visited both white and alsike clover impartially on the same trip. Philp and Vansell (19) state that the time of day influences availability of both nectar and pollen, so that activities shift during the day from one plant to the other. They have noted that filaree, in the orchards, is very attractive in the early morning, but most of the blossoms close by 10 o'clock on sunny days, and that some plants secrete nectar most abundantly during the night, the bees harvesting this in the morning and then shifting to some other plant. They declare that, in some seasons, the shift from prunes to mustard was most noticeable.

Miss Betts (5) has published results of observations on the constancy of pollen gathering bees, from microscopic examination of the loads of bees collected at the alighting board. She classifies mixed loads into two kinds; *viz.*, "S" (segregated) and "M" (mixed) mixtures. In the first case the two kinds of pollen are distinct, this type of load resulting from the bee having collected part of its load from one plant and then switched to another. In the second the two kinds are mingled and can only be recognized as a mixture under the microscope. "S" mixture constituted 40% of the 233 loads examined, but it was considered that the preponderance of "M" mixtures was much greater. Mixtures of three or more kinds only constituted 6.5% of all

the mixtures examined and contained a larger proportion of garden plants than the average (47% as against 18% for two-species mixtures). She states that her specimens were collected, "with a view to securing as many mixtures as possible, so that the percentage—6.75%—of mixed to pure loads examined is plainly much higher than it is in nature."

The well-known researches of Von Frisch (11) and Von Frisch and Rösch (12) indicate that the scout bees communicate the stimulation for pollen or nectar collecting to their followers by means of the food odor and the excitation of the "round dance" in the case of nectar collectors, odor and the "tail-wagging dance" in the case of the pollen collectors. According to these workers the scouts do not "lead" the workers to the source of food, but the latter, having once obtained the scent from the scouts, must search for it themselves. In doing so they are aided by the odors from the scent glands of the bees of the same hive visiting that particular bloom. Workers returning to the hive excite another group of workers to visit that particular plant species, either as pollen or nectar gatherers, as the case may be. Thus, as long as the supply lasts, the number of bees visiting it will increase until the maximum supply is available. Thereafter there will take place a gradual diminution in number, as the bees find the supply diminishing.

In general it may be said that the evidence from the literature seems to support the conclusion that where abundance of pasturage of a single attractive species is available bees will work that particular species almost exclusively, though they may shift from one species to another at different times of the day, when certain species are more attractive at certain definite periods. On the other hand, where a great variety of bloom is available, without a predominance of any one species, there is a greater tendency to gather mixed loads, but the evidence is conflicting as the extent to which this occurs.

Definition of Terms

From a perusal of the literature it is evident that several distinct phenomena have been included in the term "constancy" as applied to the flower-visiting habits of bees, resulting in considerable confusion. The following three types of constancy may be noted:—

Type 1. The insect confines its visits entirely to one flower species. *Type 2.* The insect confines its visits to a single species as long as it is available, but later changes to other flowers. *Type 3.* The insect confines its attention to one species of flower for each load, but may shift from one flower to the other for different loads.

The terms (1) monotrophic, (2) oligotrophic and (3) polytrophic in referring to the habit of the insect in confining its attention to (1) a single species, (2) a few related species, or (3) visiting indifferently many unrelated species, are often employed, but it should be understood that a species may be polytrophic and yet given individuals of that species may exhibit constancy of either the second or third type indicated above. Thus it is well known that

bees from the same hive may at any one time be found visiting many different species of plants. Nevertheless, this does not alter the fact that each individual bee may be confining its attention to a single species.

Constancy of the first type is well exhibited by *Halictoides novaeangliae* Robertson, which, as far as is known, confines its attention to the pickerel weed (*Pontederia cordata* L.). Constancy of the second type has been designated "polydrome constancy" by Clements and Long (8), while the term "monodrome constancy" has been proposed for the third type. Apparently some workers consider the hive bee to exemplify the second type and some the third.

Studies in the Constancy of Hive Bees, Bumble Bees and Solitary Bees to Apple Bloom

(a) General

The mechanism of constancy in solitary bees, if it exists, can hardly be identical with that in hive bees, since the form of organization of the latter is entirely different and the division of labor among workers claimed for the hive bees cannot therefore obtain among *Halicti* and *Andrenae*. However, in these studies, we are concerned with the problem of the extent to which bees in working the apple bloom, visit only that species and to what extent they carry mixed loads, also whether there is any difference in this respect among the different genera concerned in apple pollination. Whether the degree of constancy that exists is merely a matter of convenience in the greater availability of a particular species of flower, whether it is due to superior attractiveness with respect to color, odor or form at a particular time, or whether it is due to an inherited tendency or to a definite physiological response, are matters beyond the scope of this investigation. For this reason the present study is based on analyses of the pollen carried by the species concerned. It may be pointed out that where pure loads only are found, this does not necessarily prove inherent constancy, but may mean merely that at the time of collection the particular plant species was preferred because of greater availability or attractiveness of the pollen or nectar.

Furthermore, these studies refer to pollen collection only and not to nectar. Corroborative evidence from observing the visits of bees to flowers might have been desirable, but this is difficult with respect to hive bees, and still more so with solitary bees, not only on account of their small size, but on account of the rapidity of their movement. Both *Halicti* and *Andrenae* appear to fly more readily from one tree to another than do the hive bees. Both hive bees and bumble bees usually work the blossoms on a limb more consistently and tend to remain fairly long in one tree, hence their movement can, to a certain extent, be observed, but no really reliable data regarding solitary bees can be obtained in this manner.

(b) Observation Points

It will be useful in considering the results obtained in the following study to have a short description of the various "stations" from which observations were carried out.

Macdonald College. At this point there were available numerous sources of pollen in the large perennial border and surrounding wild herbaceous plants, shrubs and trees. Observations on various species of plants were made until the College orchard, which covers 30 acres, came into bloom, after which collections were confined to the apple.

Kentville, N.S. As at the preceding station a very varied source of bloom was available, but, in addition, there was a very large orchard area, 70 acres on the station property. All collections made at this station and the ones that follow, except the last, were made from the apple bloom.

Scott's Bay and North River, N.S. At these stations the orchard area was very small and other sources of pollen somewhat scant and scattered.

Long Island, N.S. Here there was a total of 96 acres of mature orchard distributed over a total land area of 640 acres. Besides cultivated land and woods there was considerable land in rough pasture containing rhododendrons, blueberries, etc.

Wolfville, N.S. Collections were made at Wolfville in early August during a period when there was a lack of abundant pasturage of any one plant, but clumps and scattered plants of Canada thistle, wild radish, fire weed, dog bane and other plants were available.

Blomidon, N.S. A large orchard area was available for pasturage at this station, in addition to which ravines and other uncultivated land furnished a limited amount of other bloom.

Material collected at other undesignated points is simply labelled "Kings Co."

(c) Method

The work was commenced at Macdonald College early in May when the early plants in the perennial borders were in bloom, but before apple bloom was available. Pollen was first obtained from all available bloom and mounted on slides for comparison with material to be later taken from the bees. This was kept up throughout the period of the study, samples of pollen from new plants being obtained and examined as fast as they came into blossom. In this way it was possible to determine most of the different kinds of pollen, at least as far as the genus. Previous to the blossoming of the apple, bees were collected from all available bloom, but after the apple bloom appeared, all bee collections were confined to it, and all available species were collected. Pollen was removed from the body hairs with a scalpel, the process being usually carried out under a dissecting microscope. Temporary or permanent mounts were then made for later study and photographing.

While collections of male bees were made and analyses of their pollen loads performed, the analysis of the data disregards entirely collections made from the males, owing to the inconstancy of the males of many species, in which a considerable degree of constancy is exhibited by the females.

It should be emphasized that the true constancy of the species studied is not necessarily clearly indicated by the analyses presented, because they do

not show the proportion of foreign pollen to host pollen. In many cases, however, the collections, especially those from apple bloom, show a large proportion of the individual loads to belong to a single plant species. Hence, the actual constancy of all species as far as single loads are concerned, may be actually considerably greater than indicated by percentage figures of pure and mixed loads.

It should be further explained that, in these studies, special attention was given to the pollen adhering to the body hairs, since it was considered that this would have the most significance in pollination and it could readily be washed from the body with alcohol. By following this method, large samples were not obtained, but mixtures, when they occurred, were readily recognizable. On the other hand, mixtures of pollen from the corbiculae of hive bees or bumble bees may be difficult to detect, especially if they are of the "mixed" type mentioned by Miss Betts. The "segregated" type can be readily detected if the pollen masses are of different color. The manner of securing our samples for analyses may possibly account for the fact that a higher percentage of mixtures was found than some other workers have reported. One would expect less error from contamination than would occur were the bees caught at the hive entrance. Possibly some of the pollen reported, such as that of *Phleum pratense*, may represent an accidental contamination, as we have never noted bees visiting this plant. Nevertheless, it is well known that, at certain times, bees will deliberately collect all sorts of material including flour or even sawdust and it may be that the pollen was actually gathered by the bees.

(d) Host Plants

In the accompanying tables only the genus of the host species and the pollen species is given in most cases. This is partly for the purpose of brevity in compiling the tables and partly because our main interest was merely to establish whether the insects concerned carried pure or mixed loads, which is sufficiently indicated by the foregoing practice. When a bee was taken on a certain host and the pollen taken from the body hairs was obviously of the same genus, the presumption would be that it was collected on that particular host, but when a pollen mixture was obtained it was not always possible to be sure of the exact species of the foreign pollen, owing to the close similarity between pollen of different members of the same genus. If the bee was taken from apple and bore, in addition to apple pollen, that of *Vaccinium* or *Trifolium*, it was not possible to distinguish between the species of the foregoing genera. Neither was it necessary in order to classify the sample as a "mixed" load. In the case of *Taraxacum officinale* our task was easy, as the pollen of this plant does not resemble that of any other blossoming at the same time. In other cases, as with liliaceous hosts, it is even difficult to separate genera.

Accordingly, the following list shows only the plant species from which bees were actually collected.

TABLE I

LIST OF HOST PLANTS ON WHICH COLLECTIONS WERE MADE

Scientific name	Common name	Place of collection
Acer platanoides L.	Norway maple	Macdonald College, P.Q.
Alyssum saxatile L.	Alyssum	Kentville, N.S.
Amelanchier canadensis L.	June berry	Macdonald College, P.Q.
Brassica oleraceae L.	Cabbage	Kings Co., N.S.
Caragana arborescens Lam.	Siberian pea tree	Macdonald College, P.Q.
Cerastium tomentosum L.	Snow-in-summer	Macdonald College, P.Q.
Chrysanthemum leucanthemum L.	Ox-eye daisy	North River; Blomidon.
Cornus canadensis L.	Bunchberry	Blomidon; North River.
Cirsium arvense (L.) Scip.	Canada thistle	Wolfville, N.S.
Daucus carota L.	Wild carrot	Blomidon; Scott's Bay.
Fragaria virginiana (Duchesne)	Wild strawberry	Kentville, N.S.
Leontodon autumnalis L.	Fall dandelion	Wolfville, N.S.
Lonicera tartarica L.	Tartarian honeysuckle	Macdonald College, P.Q.
Muscari botryoides L.	Grape hyacinth	Macdonald College, P.Q.
Narcissus pseudo-narcissus L.	Daffodil	Macdonald College, P.Q.
Narcissus poeticus L.	Poet's narcissus	Macdonald College, P.Q.
Prunus avium L.	Cultivated sweet cherry	Macdonald College, P.Q.
Prunus nigra Ait.	Wild or Canada plum	Macdonald College, P.Q.
Pyrus malus (Hill) S. F. Gray	Apple	Kentville; Long Island.
Rosa spp.	Wild rose	Wolfville, N.S.
Rubus spp.	Blackberry	Kings Co., N.S.
Raphanus raphanistrum L.	Wild radish	Wolfville, N.S.
Rhododendron canadense (L.) B.S.P.	Rhodora	Kentville; Long Island.
Salix incana Schank	Willow	Macdonald College, P.Q.
Sambucus aurea Cowell	Golden elder	Kentville, N.S.
Scilla sibirica Andr.	Siberian squill	Macdonald College, P.Q.
Solidago canadensis L.	Goldenrod	Wolfville, N.S.
Spiraea vanhouttei Zabel	Spiraea vanhouttei	Macdonald College, P.Q.
Taraxacum officinale Weber	Common dandelion	All stations.
Trifolium repens L.	White clover	North River; Blomidon; Scott's Bay.
Tulipa gesneriana L.	Tulip	Macdonald College, P.Q.
Vaccinium canadense Kalm.	Blueberry	Blomidon; Scott's Bay.
V pennsylvanicum Lam.	Blueberry	North River; Macdonald College, P.Q.

In Table II are listed the various species collected and the result of analysis of their pollen loads from all the stations. In cases in which a bee was taken on a certain plant, but bore pollen from another, we have counted this a "mixed visit," on the assumption that it had not yet had time to secure pollen from the plant on which it was found, in sufficient quantity to appear in the analyses.

TABLE II

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species
Andrena crataegi Robt.—Macdonald College			
May 16	2	*Prunus*	*Prunus; Taraxacum; Caragana*
May 16	2	*Prunus*	*Prunus; Caragana*
May 16	4	*Prunus*	*Prunus*
May 16	2	*Prunus*	*Prunus; Taraxacum; Tulipa*
May 16	1	*Prunus*	*Prunus; Taraxacum*
May 16	2	*Prunus*	*Prunus; Spiraea; Taraxacum*
May 19	1	*Spiraea*	*Taraxacum*
May 19	1	*Spiraea*	*Spiraea; Prunus; Tulipa*
May 19	1	*Spiraea*	*Spiraea; Tulipa*
May 19	1	*Narcissus*	*N. pseudo-narcissus; Tulipa; Salix*
May 19	2	*Narcissus*	*N. pseudo-narcissus; Tulipa*
May 19	2	*Narcissus*	*Tulipa; Salix*
May 19	3	*Narcissus*	*Tulipa*
May 19	1	*Narcissus*	*Prunus; Salix*
May 19	1	*Amelanchier*	*Taraxacum; Amelanchier; Spiraea*
May 19	3	*Spiraea*	*Spiraea; Tulipa; N. pseudo-narcissus*
May 19	1	*Spiraea*	*Spiraea; Taraxacum; Tulipa; N. pseudo-narcissus*
May 19	2	*Spiraea*	*Spiraea; Tulipa; Taraxacum*
May 19	1	*Spiraea*	*Spiraea; Prunus*
May 19	1	*Spiraea*	*Spiraea; Caragana; Prunus*
May 19	1	*Spiraea*	*Spiraea; Prunus; N. pseudo-narcissus*
Andrena crataegi Robt.—North River			
June 9	1	*Pyrus malus*	*Vaccinium; Trifolium*
Andrena crataegi Robt.—Blomidon			
June 13.	1	*Pyrus malus*	*Pyrus malus*
Andrena annae Ckll.—Macdonald College			
May 19	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
Andrena carlini Ckll.—Kentville			
May 25	1	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Pyrus malus; Tragana*
May 30	2	*Pyrus malus*	*Pyrus malus*
Andrena carlini Ckll.—Blomidon			
June 12	3	*Pyrus malus*	*Pyrus malus*
June 12	2	*Pyrus malus*	*Pyrus malus; Trifolium*
June 13	2	*Pyrus malus*	*Pyrus malus*
Andrena sp.—Kentville			
May 25	7	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Sambucus*
May 25	1	*Pyrus malus*	*Taraxacum*
Andrena bradleyi Vier—Kentville			
May 30	1	*Pyrus malus*	*Pyrus malus; Rhododendron*
Andrena milwaukieensis—Kentville			
June 4	1	*Pyrus malus*	*Pyrus malus*
Andrena obscura Robt.—Macdonald College			
May 9	2	*Salix*	*Salix*
May 12	1	*Salix*	*Tulipa*
May 19	1	*Pyrus malus*	*Pyrus malus*
June 9	1	*Pyrus malus*	*Vaccinium*
June 9	1	*Pyrus malus*	*Trifolium*
Andrena rugosa Robt.—Scott's Bay			
June 16	1	*Pyrus malus*	*Pyrus malus*
June 16	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
Andrena thaspii Graen.—North River			
June 9	7	*Pyrus malus*	*Vaccinium*
June 9	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
June 9	2	*Pyrus malus*	*Pyrus malus; Vaccinium*
June 9	2	*Pyrus malus*	*Vaccinium; Trifolium*
June 9	3	*Pyrus malus*	*Pyrus malus; Chrysanthemum*
June 9	1	*Pyrus malus*	*Chrysanthemum*
June 9	4	*Pyrus malus*	*Pyrus malus*
Andrena wilkella Kirby—North River			
June 9	1	*Pyrus malus*	*Pinus*
Andrena wilkella Kirby—Long Island			
June 9	5	*Pyrus malus*	*Pyrus malus*
Andrena wilkella Kirby—Blomidon			
June 12	1	*Pyrus malus*	*Pyrus malus*
June 12	3	*Pyrus malus*	*Pyrus malus*
June 12	1	*Pyrus malus*	*Pyrus malus; Daucus carota*
Andrena wilkella Kirby—Scott's Bay			
June 12	30	*Pyrus malus*	*Pyrus malus*
June 12	12	*Pyrus malus*	*Pyrus malus; Taraxacum*
June 12	1	*Pyrus malus*	*Pyrus malus; Trifolium*
Andrena wilkella Kirby—Wolfville			
July 30	1	*Cirsium*	*Cirsium*
Aug. 1	3	*Solidago*	*Solidago*
Andrena sp.—Macdonald College			
May 9	1	*Salix*	*Salix; N. pseudo-narcissus*
May 12	2	*Salix*	*Salix; Tulipa*
May 12	1	*Salix*	*Tulipa*
May 16	1	*Acer*	*Tulipa*
May 16	1	*Prunus*	*Prunus; Caragana*
May 16	1	*Prunus*	*Prunus; Taraxacum; Phlox sublata*
May 16	1	*Prunus*	*Prunus; Taraxacum*
May 19	2	*Pyrus malus*	*Pyrus malus*
May 19	1	*Pyrus malus*	*Pyrus malus; Lonicera*
May 19	1	*Spiraea*	*Tulipa*

(*) *Rhododendron predominating.*

TABLE II—*Continued*

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species
***Andrena* sp.—Kentville**			
May 30	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
***Andrena* sp.—Macdonald College**			
June 1	1	*Taraxacum*	*Taraxacum*
June 1	1	*Taraxacum*	*Taraxacum; Pyrus malus*
June 1	1	*Taraxacum*	*Taraxacum; Caragana*
***Andrena* sp.—North River**			
June 9	1	*Pyrus malus*	*Pyrus malus; Vaccinium; Trifolium Chrysanthemum*
June 9	1	*Pyrus malus*	*Pyrus malus; Vaccinium*
June 9	1	*Pyrus malus*	*Vaccinium*
June 9	1	*Pyrus malus*	*Pyrus malus*
***Andrena* sp.—Scott's Bay**			
June 16	2	*Pyrus malus*	*Pyrus malus*
June 16	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
***Andrena vicina* Smith—Macdonald College**			
May 9	1	*Scilla*	*Scilla; Taraxacum*
May 16	1	*Prunus*	*Prunus; Taraxacum; Caragana*
May 16	1	*Prunus*	*Prunus; Taraxacum; Vaccinium*
May 19	2	*Pyrus malus*	*Pyrus malus*
May 19	1	*Pyrus malus*	*Pyrus malus; Prunus*
May 19	1	*Pyrus malus*	*Pyrus malus; Taraxacum; Tulipa*
May 19	1	*Spiraea*	*Spiraea; Prunus; Tulipa*
May 31	1	*Prunus*	*Prunus; Taraxacum*
***Andrena vicina* Smith—North River**			
June 9	2	*Pyrus malus*	*Pyrus malus*
June 9	1	*Pyrus malus*	*Pyrus malus; Trifolium*
***Apis mellifica* L.—Macdonald College**			
May 9	1	*Salix*	*Salix; Taraxacum* (*)
May 9	3	*Salix*	*Salix*
May 9	1	*Narcissus*	*N. pseudo-narcissus*
May 12	1	*Salix*	*Salix; N. poeticus*
May 12	2	*Salix*	*Taraxacum; N. pseudo-narcissus; Salix*
May 12	7	*Salix*	*Salix; N. pseudo-narcissus*
May 12	8	*Salix*	*Salix; Tulipa*
May 12	7	*Salix*	*Salix; N. pseudo-narcissus; Tulipa*
May 12	4	*Salix*	*Tulipa*
May 12	1	*Salix*	*N. pseudo-narcissus; Tulipa*
May 12	2	*Salix*	*Salix; Tulipa; Taraxacum*
Apis mellifica* L.—Macdonald College—*Concluded			
May 12	1	*Salix*	*N. pseudo-narcissus*
May 16	1	*Taraxacum*	*Taraxacum; N. pseudo-narcissus*
May 16	2	*Taraxacum*	*Taraxacum; Tulipa*
May 16	2	*Taraxacum*	*Taraxacum; Tulipa; N. pseudo-narcissus*
May 16	1	*Taraxacum*	*Taraxacum; Salix*
May 16	1	*Taraxacum*	*Taraxacum; Tulipa; Salix*
May 16	2	*Taraxacum*	*N. pseudo-narcissus; Tulipa; Taraxacum; Salix*
May 16	1	*Taraxacum*	*N. pseudo-narcissus; Crocus; Taraxacum; Tulipa; Salix*
May 16	1	*Taraxacum*	*Salix; Tulipa*
May 16	1	*Taraxacum*	*Salix; N. pseudo-narcissus; Tulipa*
May 16	1	*Taraxacum*	*Tulipa* (†)
May 16	1	*Prunus*	*Prunus; Tulipa; Lonicera*
May 16	1	*Prunus*	*Tulipa; Prunus*
May 16	1	*Prunus*	*Prunus; Caragana; Phlox subulata*
May 16	1	*Prunus*	*Prunus; Caragana*
May 16	1	*Acer*	*Tulipa*
May 16	1	*Acer*	*Tulipa*
May 16	1	*Acer*	*Tulipa*
May 19	2	*Prunus*	*Prunus; Taraxacum* (‡)
May 19	1	*Prunus*	*Prunus; Taraxacum; Lonicera*
May 19	1	*Prunus*	*Prunus; Caragana*
May 19	1	*Narcissus*	*Tulipa*
May 19	2	*Pyrus malus*	*Pyrus malus*
May 19	2	*Pyrus malus*	*Pyrus malus; Taraxacum*
May 19	3	*Spiraea*	*Spiraea; Prunus*
May 29	7	*Pyrus malus*	*Pyrus malus*
May 29	1	*Pyrus malus*	*Pyrus malus; Tulipa*
May 31	1	*Prunus*	*Prunus; Taraxacum*
May 31	3	*Pyrus malus*	*Pyrus malus*
May 31	1	*Pyrus malus*	*Pyrus malus; Caragana*
May 31	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
June 1	4	*Taraxacum*	*Taraxacum*
June 1	7	*Taraxacum*	*Taraxacum; Pyrus malus*
June 2	8	*Pyrus malus*	*Pyrus malus*
June 2	3	*Pyrus malus*	*Pyrus malus; Taraxacum*

(*) *Pollen scarce; bees collected on (Salix) pistillate tree. Staminate tree in full bloom.*
(†) *Pollen packed.*
(‡) *Collected before full apple bloom.*

TABLE II—*Continued*

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species
Apis mellifica **L.—Kentville**			
May 25	2	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Pyrus malus; Taraxacum* (*)
May 30	43	*Pyrus malus*	*Pyrus malus*
May 30	7	*Pyrus malus*	*Pyrus malus; Taraxacum* (†)
Apis mellifica **L.—Long Island**			
June 4	25	*Pyrus malus*	*Pyrus malus*
June 9	21	*Pyrus malus*	*Pyrus malus*
Apis mellifica **L.—North River**			
June 9	1	*Pyrus malus*	*Pyrus malus; Vaccinium; Trifolium*
June 11	2	*Pyrus malus*	*Pyrus malus; Vaccinium*
June 11	1	*Pyrus malus*	*Pyrus malus*
June 11	1	*Pyrus malus*	*Pyrus malus, Vaccinium; Cornus canadensis*
June 11	1	*Pyrus malus*	*Pyrus malus; Vaccinium; Cornus canadensis; Phleum*
June 12	5	*Pyrus malus*	*Pyrus malus; Vaccinium*
June 12	1	*Pyrus malus*	*Pyrus malus; Vaccinium; Chrysanthemum*
June 12	2	*Pyrus malus*	*Pyrus malus; Chrysanthemum*
June 12	1	*Pyrus malus*	*Pyrus malus*
Apis mellifica **L.—Blomidon**			
June 13	5	*Pyrus malus*	*Pyrus malus*
Apis mellifica **L.—Wolfville**			
July 30	9	*Cirsium*	*Cirsium*
Aug. 1	3	*Solidago*	*Solidago*
Aug. 1	1	*Rosa*	*Rosa; Leontodon*
Aug. 10	22	*Raphanus*	*Raphanus*
Aug. 10	1	*Raphanus*	*Raphanus; Leontodon*
Aug. 10	3	*Solidago*	*Solidago*
Apis mellifica **L.—Scott's Bay**			
June 16	10	*Pyrus malus*	*Pyrus malus*
Bremus borealis **Kirby—Scott's Bay**			
June 16	2	*Pyrus malus*	*Pyrus malus*
Bremus fervidus **Fob.—Macdonald College**			
May 12	1	*Narcissus*	*N. pseudo-narcissus; Tulipa*
May 12	1	*Narcissus*	*N. pseudo-narcissus; Tulipa; Salix; Taraxacum*
May 12	1	*Narcissus*	*Taraxacum; Tulipa; N. pseudo-narcissus*
May 9	1	*Narcissus*	*N. pseudo-narcissus*
May 9	1	*Muscari*	*Muscari*
May 19	1	*Amelanchier*	*Amelanchier; Tulipa*
Bremus fervidus **Fob.—Macdonald College—*Concluded***			
May 25	2	*Lonicera*	*Lonicera; Taraxacum; Caragana*
May 25	1	*Lonicera*	*Lonicera*
Bremus fervidus **Fob.—Blomidon**			
June 12	1	*Pyrus malus*	*Pyrus malus; Phleum; Daucus carota*
June 13	1	*Pyrus malus*	*Pyrus malus; Daucus carota*
June 16	3	*Pyrus malus*	*Pyrus malus; Phleum*
June 16	2	*Pyrus malus*	*Pyrus malus*
Bremus fervidus **Fob.—Scott's Bay**			
June 16	2	*Pyrus malus*	*Phleum*
Bremus ternarius **Say.—Macdonald College**			
May 12	1	*Narcissus*	*N. pseudo-narcissus; Tulipa*
June 2	1	*Pyrus malus*	*Pyrus malus*
Bremus ternarius **Say.—Kentville**			
May 25	2	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Pyrus malus; Rhododendron*
June 4	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
Bremus ternarius **Say.—Long Island**			
May 25	1	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Pyrus malus; Rhododendron*
June 4	1	*Pyrus malus*	*Pyrus malus; Rhododendron*
Bremus ternarius **Say.—Blomidon**			
June 12	1	*Pyrus malus*	*Pyrus malus; Chrysanthemum*
June 13	1	*Pyrus malus*	*Pyrus malus; Daucus carota*
Bremus ternarius **Say.—Scott's Bay**			
June 16	8	*Pyrus malus*	*Pyrus malus*
June 16	1	*Pyrus malus*	*Pyrus malus; Pinus*
June 16	1	*Pyrus malus*	*Vaccinium*
Bremus ternarius **Say.—Wolfville**			
July 30	1	*Cirsium*	*Cirsium*
Aug. 1	1	*Solidago*	*Cirsium*
Bremus terricola **Kirby—Macdonald College**			
May 9	1	*Salix*	*Salix*
May 25	1	*Lonicera*	*Lonicera*
Bremus terricola **Kirby—Blomidon**			
June 13	1	*Pyrus malus*	*Pinus; Phleum*
Bremus terricola **Kirby—Scott's Bay**			
June 13	3	*Pyrus malus*	*Pyrus malus*
Bremus vagans **Smith—Macdonald College**			
May 9	1	*Salix*	*Salix; N. pseudo-narcissus*
May 9	1	*Salix*	*Taraxacum*
May 9	1	*Salix*	*Salix*
May 19	1	*Spiraea*	*Spiraea; Prunus*
May 21	1	*Caragana*	*Caragana; Tulipa*

(*) *Pyrus predominating.*
(†) *Three with Pyrus predominating.*

TABLE II—*Continued*

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species
Bremus vagans Smith—North River			
July 9	2	*Pyrus malus*	*Pyrus malus*
Bremus vagans Smith—Scott's Bay			
May 25	1	*Pyrus malus*	*Pyrus malus*
June 16	18	*Pyrus malus*	*Pyrus malus*
June 16	1	*Pyrus malus*	*Phleum; Pinus*
June 16	1	*Pyrus malus*	*Pinus*
June 16	1	*Pyrus malus*	*Pyrus malus; Vaccinium*
June 16	1	*Pyrus malus*	*Vaccinium*
June 16	1	*Pyrus malus*	*Pyrus malus; Vaccinium; Phleum*
June 16	1	*Pyrus malus*	*Pyrus malus; Daucus carota*
Bremus sp.—Macdonald College			
May 19	1	*Spiraea*	*Spiraea; Prunus; Taraxacum*
May 20	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
May 20	1	*Pyrus malus*	*Pyrus malus*
May 29	1	*Prunus*	*Prunus*
Bremus sp.—Blomidon			
June 12	1	*Pyrus malus*	*Pyrus malus; Phleum; Vaccinium; Chrysanthemum*
Halictus arcuatus Robt.—Kentville			
May 25	5	*Pyrus malus*	*Pyrus malus*
May 25	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
Halictus craterus Lov.—North River			
June 9	1	*Pyrus malus*	*Chrysanthemum; Trifolium*
June 11	1	*Pyrus malus*	*Pyrus malus*
Halictus craterus Lov.—Wolfville			
July 30	31	*Cirsium*	*Cirsium*
July 30	1	*Cirsium*	*Cirsium; Lychnis*
Halictus craterus Lov.—Wolfville			
Aug. 6	107	*Leontodon*	*Leontodon*
Aug. 6	1	*Rosa*	*Rosa; Leontodon;* unknown sp. (*)
Aug. 6	1	*Rosa*	*Leontodon;* unknown sp. (*)
Aug. 6	1	*Rosa*	*Leontodon; Rosa* (*)
Aug. 6	3	*Rosa*	*Rosa*
Halictus cressonii Robt.—Blomidon			
June 11	1	*Pyrus malus*	*Pyrus malus; Phleum*
Halictus cressonii Robt.—Scott's Bay			
June 16	4	*Pyrus malus*	*Pyrus malus*
Halictus cressonii Robt.—Kings Co.			
June, 1932	4	*Brassica*	*Brassica*
June, 1932	1	*Rhododendron*	*Rhododendron; Pyrus malus* (†)
Halictus coriaceus Smith—Scott's Bay			
June 16	1	*Pyrus malus*	*Pyrus malus*
Halictus foxii Robt.—Kentville			
May 25	1	*Pyrus malus*	*Pyrus malus*
Halictus foxii Robt.—Macdonald College			
June 1	1	*Taraxacum*	*Taraxacum*
Halictus foxii Robt.—North River			
June 9	1	*Pyrus malus*	*Vaccinium*
June 9	1	*Pyrus malus*	*Pyrus malus; Trifolium*
June 11	1	*Pyrus malus*	*Pyrus malus; Trifolium*
Halictus foxii Robt.—Blomidon			
June 11	1	*Pyrus malus*	*Pyrus malus; Phleum*
Halictus foxii Robt.—Scott's Bay			
June 16	1	*Pyrus malus*	*Pyrus malus*
Halictus foxii Robt.—Wolfville			
June, 1932	1	*Rubus*	*Rubus*
June, 1932	1	*Brassica*	*Brassica*
June, 1932	1	*Rubus*	*Rubus;* unknown sp. (‡)
Halictus lerouxii LeP.—Macdonald College			
May 12	1	*Salix*	*Tulipa*
Halictus lerouxii LeP.—Kentville			
May 25	2	*Pyrus malus*	*Pyrus malus*
Halictus lerouxii LeP.—Long Island			
June 4	1	*Pyrus malus*	*Pyrus malus*
Halictus lerouxii LeP.—Blomidon			
June 13	1	*Pyrus malus*	*Pyrus malus*
Halictus lerouxii LeP.—Scott's Bay			
June 16	1	*Pyrus malus*	*Pyrus malus*
Halictus lerouxii LeP.—Wolfville			
Aug. 1	1	*Solidago*	*Solidago*
Halictus macoupinensis—Kings Co.			
June, 1932	2	*Rubus*	*Rubus*
June, 1932	3	*Brassica*	*Brassica*
June, 1932	1	*Rubus*	*Rubus;* unknown sp.
Halictus pectoralis—Kings Co.			
Aug. 1	1	*Solidago*	*Solidago*
Halictus pilosus Smith—Kentville			
May 25	1	*Pyrus malus*	*Pyrus malus; Taraxacum*
Halictus pilosus Smith—Kings Co.			
June, 1932	1	*Brassica*	*Brassica*
Halictus provancheri D. T.—Macdonald College			
May 9	1	*Salix*	*Salix*
May 9	1	*Cerastium*	*Cerastium*
May 9	4	*Scilla*	*Scilla*
Halictus provancheri D. T.—Scott's Bay			
June 16	1	*Pyrus malus*	*Pyrus malus; Trifolium*
Halictus provancheri D. T.—Wolfville			
July 30	1	*Cirsium*	*Cirsium*
Halictus provancheri D. T.—Kings Co.			
June, 1932	1	*Rubus*	*Rubus;* unknown sp.
June, 1932	1	*Brassica*	*Brassica;* unknown sp.

(*) ***Leontodon predominating.***
(†) ***Pyrus predominating.***
(‡) ***Rubus predominating.***

TABLE II—*Concluded*

LIST OF BEES—CLASSIFIED ACCORDING TO SPECIES

Date	No. examined	Host	Pollen species
Halictus smilacinae **Robt.—Kentville**			
May 25	14	*Pyrus malus*	*Pyrus malus*
May 25	6	*Pyrus malus*	*Pyrus malus; Taraxacum* (*)
Halictus smilacinae **Robt.—Long Island**			
June 4	2	*Pyrus malus*	*Pyrus malus*
Halictus smilacinae **Robt.—Scott's Bay**			
June 9	2	*Pyrus malus*	*Pyrus malus*
Halictus smilacinae **Robt.—Blomidon**			
June 11	1	*Pyrus malus*	*Pyrus malus; Trifolium; Vaccinium*
June 11	1	*Pyrus malus*	*Pyrus malus*
June 16	1	*Pyrus malus*	*Pyrus malus*
Halictus smilacinae **Robt.—Wolfville**			
July 30	1	*Cirsium*	*Cirsium*
Aug. 1	6	*Solidago*	*Solidago*
Aug. 1	1	*Solidago*	*Solidago; Rosa*
Aug. 1	2	*Solidago*	*Solidago;* unknown sp.
Halictus smilacinae **Robt.—Kings Co.**			
June, 1932	1	*Brassica*	*Brassica;* 2 unknown sp.
June, 1932	3	*Brassica*	*Brassica*
June, 1932	3	*Brassica*	*Brassica;* 1 unknown sp.
June, 1932	2	*Brassica*	Unknown sp.
June, 1932	1	*Taraxacum*	*Taraxacum*
June, 1932	1	*Prunus*	*Prunus*
Halictus **sp.—Macdonald College**			
May 9	2	*Scilla*	*Scilla*
May 9	1	*Muscari*	*Tulipa*
May 9	1	*Salix*	*Salix*
May 9	1	*Cerastium*	*Cerastium*
May 9	2	*Taraxacum*	*Taraxacum*
Halictus **sp.—Macdonald College—*Concluded***			
May 12	1	*Narcissus*	*N. pseudo-narcissus*
May 12	1	*Narcissus*	*Tulipa*
May 12	1	*Narcissus*	*N. pseudo-narcissus; Tulipa; Salix*
May 19	1	*Amelanchier*	*Spiraea; Amelanchier*
June 1	1	*Taraxacum*	*Taraxacum*
June 1	1	*Taraxacum*	*Taraxacum; Caragana*
June 16	2	*Pyrus malus*	*Pyrus malus*
Halictus **sp.—Kentville**			
May 25	1	*Pyrus malus*	*Pyrus malus*
Ceratina **sp.—Macdonald College**			
May 9	1	*Amelanchier*	*Amelanchier*
Colletes **sp.—Wolfville**			
Aug. 1	4	*Solidago*	*Solidago*
Aug. 1	1	*Solidago*	*Solidago; Rosa*
Megachile **sp.—Scott's Bay**			
June 16	1	*Pyrus malus*	*Pyrus malus*
Megachile **sp.—Wolfville**			
July 30	1	*Cirsium*	*Cirsium; Raphanus*
July 30	1	*Cirsium*	*Cirsium*
July 30	1	*Solidago*	*Solidago*
Mellisoides **sp.—Wolfville**			
Aug. 1	2	*Solidago*	*Solidago*
Aug. 1	1	*Solidago*	*Solidago; Rosa*
Aug. 6	1	*Leontodon*	*Leontodon*
Osmia **sp.—Macdonald College**			
June 9	1	*Pyrus malus*	*Trifolium*
June 9	1	*Pyrus malus*	*Pyrus malus; Trifolium*
June 9	1	*Pyrus malus*	*Vaccinium; Pyrus malus; Chrysanthemum*
***Specbodes*—Macdonald College**			
June 9	1	*Pyrus malus*	*Vaccinium*

(*) *Pyrus predominating in all but one case.*

Based on the foregoing, Table III has been prepared in which have been tabulated (1) the totals of pure and mixed loads collected from all species of plants by representatives of the four genera, *Andrena*, *Apis*, *Bremus*, and *Halictus*, (2) the summarized results from all stations applied to apple visitors only, (3) the results from mixed collections at Macdonald College previous to bloom, (4) the separate results for apple visitors only from Macdonald College, Kentville, Long Island, Scott's Bay and Blomidon, and (5) the results from mixed collections at Wolfville made during the early part of August.

While still larger numbers of individual collections would have been preferable, particularly during apple bloom, the data, thus made available, do furnish useful information regarding the comparative constancy of the species taken under varied conditions.

TABLE III

FLOWER CONSTANCY OF HIVE AND WILD BEES

Genus	Observation station	Host plant	Pure loads		Mixed visits				Total
			No.	%	2 spp.	3 spp.	4 spp.	5 spp.	
Andrena	All stations	All species	82	45.50	70	26	2	—	98
Apis	All stations	All species	163	61.97	73	22	4	1	100
Bremus	All stations	All species	50	58.80	25	8	2	—	35
Halictus	All stations	All species	207	83.90	34	6	—	—	40
Andrena	All stations	*Pyrus malus*	71	57.20	47	5	1	—	53
Apis	All stations	*Pyrus malus*	118	80.30	25	3	1	—	29
Bremus	All stations	*Pyrus malus*	41	65.00	17	4	1	—	22
Halictus	All stations	*Pyrus malus*	41	71.90	14	2	—	—	16
Andrena	Macdonald College	Mixed species	15	21.70	28	24	2	—	54
Apis	Macdonald College	Mixed species	28	26.60	55	18	3	1	77
Bremus	Macdonald College	Mixed species	19	59.40	88	4	—	1	13
Halictus	Macdonald College	Mixed species	18	81.80	4	—	—	—	4
Andrena	Macdonald College	*Pyrus malus*	6	60.0	3	1	—	—	4
Apis	Macdonald College	*Pyrus malus*	20	71.4	8	—	—	—	8
Bremus	Macdonald College	*Pyrus malus*	2	66.6	1	—	—	—	1
Halictus	Macdonald College	*Pyrus malus*	—	—	—	—	—	—	—
Andrena	Kentville	*Pyrus malus*	11	68.75	5	—	—	—	5
Apis	Kentville	*Pyrus malus*	45	84.9	8	—	—	—	8
Bremus	Kentville	*Pyrus malus*	3	60.0	2	—	—	—	2
Halictus	Kentville	*Pyrus malus*	24	77.4	7	—	—	—	7
Andrena	Long Island	*Pyrus malus*	5	100.0	—	—	—	—	—
Apis	Long Island	*Pyrus malus*	46	100.0	—	—	—	—	—
Bremus	Long Island	*Pyrus malus*	1	33.3	2	—	—	—	2
Halictus	Long Island	*Pyrus malus*	3	100.0	—	—	—	—	—
Andrena	North River	*Pyrus malus*	6	21.4	18	3	1	—	22
Apis	North River	*Pyrus malus*	2	14.3	9	2	1	—	12
Bremus	North River	*Pyrus malus*	2	100.0	—	—	—	—	—
Halictus	North River	*Pyrus malus*	1	25.0	3	1	—	—	4
Andrena	Scott's Bay	*Pyrus malus*	33	67.4	16	—	—	—	16
Apis	Scott's Bay	*Pyrus malus*	10	100.0	—	—	—	—	—
Bremus	Scott's Bay	*Pyrus malus*	32	76.3	9	1	—	—	10
Halictus	Scott's Bay	*Pyrus malus*	9	90.0	1	—	—	—	1
Andrena	Blomidon	*Pyrus malus*	11	78.6	3	—	—	—	3
Apis	Blomidon	*Pyrus malus*	5	100.0	—	—	—	—	—
Bremus	Blomidon	*Pyrus malus*	2	16.7	6	3	1	—	10
Halictus	Blomidon	*Pyrus malus*	3	37.5	2	2	1	—	5
Andrena	Wolfville	All species	4	100.0	—	—	—	—	—
Apis	Wolfville	All species	37	95.0	2	—	—	—	2
Bremus	Wolfville	All species	2	100.0	—	—	—	—	—
Halictus	Wolfville	All species	150	88.8	16	3	—	—	19

Comments on Results

It would appear from the results from all stations that, considering the high degree of error inherent in calculations based on such comparatively small numbers, none of the bee species show as high a degree of constancy as some workers have claimed, when the records from all flowers visited are

studied. The difference between the genera when considered in this way, is not particularly significant. Considering the data from all stations and from all host species *Halictus* shows the highest degree of constancy, followed by *Apis*, *Bremus* and *Andrena*. Considering only the data secured from material collected during apple bloom, *Apis* appears relatively more constant and *Halictus* somewhat less so, while following them in order we have *Bremus* and *Andrena*. In all cases the different species show 50% or more of the individuals collected carrying pure loads. Considering only results from the great variety of bloom available at Macdonald College, *Halictus* shows greatest constancy and *Andrena* least, with *Bremus* and *Apis* in order following an intermediate position. At Wolfville, also from mixed bloom and neglecting *Bremus* and *Andrena* because of the insignificant number collected, we have *Apis* again taking first place, followed by *Halictus*, both showing a high degree of constancy.

The figures from the various individual stations are too small, especially in the case of wild bees, to draw any sweeping conclusions, but they indicate, with one unimportant exception, that, when abundant apple bloom is available, all species show a degree of constancy exceeding 50% and sometimes reaching 100%. The figures do not permit us to conclude with any degree of certainty that any of the species studied show any decided advantage over the others in regard to constancy, except that *Apis* and *Halictus* appear, on the whole to have an advantage. When abundant bloom of many different species was available, as at Macdonald College, without a predominance of any one plant species, the constancy exhibited was generally less for all genera. Though records at Wolfville were taken from mixed bloom the conditions were somewhat different to those at Macdonald College, since the number of plant species available was less. This is reflected in results obtained, which show a much greater degree of constancy than in the spring counts. At this station we have significant numbers only for *Halictus* and *Apis*, both showing a high degree of constancy, with the margin in favor of the latter.

It would thus appear that the degree of constancy exhibited by the insects concerned varies considerably with the situation, availability and attractiveness being important factors. Where large exposures of bloom of an attractive species are available all forms studied appear to prefer to follow a single host. The degree of constancy became less, the greater the multiplicity of hosts, with an insufficiency of any particular one to furnish adequate pasturage. It would also appear that, by selecting particular periods of the year and a particular host distribution, one could obtain almost any result desired, with respect to the constancy of any species.

Summary

1. This investigation was undertaken primarily to determine the relative pollen constancy of hive bees and wild bees to apple bloom and, secondarily, to various spring and summer blooming plants.

2. The observations were made at Macdonald College, Que., and at various stations in Kings Co., Nova Scotia.

3. Pollen for analysis was obtained both from the body hairs and from the corbiculae of the bees.

4. Previous to apple bloom collections of bees were made on a wide range of hosts. During the apple blossoming period collections were confined to that species. Late summer collections were made mainly from the Compositae.

5. Constancy in all species bore a direct relationship to the relative abundance of different types of bloom.

6. Generic differences in constancy were not particularly significant. During the period of apple bloom *Apis* appeared to be the most constant, but through the entire season the *Halicti* exhibited the highest average constancy.

7. In the pollen analyses, the species present were recorded without estimation of the relative amount of each kind represented. Had this been done the degree of constancy for all species would have appeared higher.

Acknowledgments

Miss Janie Matheson carried out the details of the work of pollen analyses at Macdonald College and a part of those in Nova Scotia. Mr. Robert Ward and Mr. John Leefe carried out a large proportion of the analyses made in Nova Scotia. Professor J. G. Coulson, Macdonald College, provided laboratory space and gave freely of his time in connection with the photographing of the pollen samples taken at Macdonald College. Identification of the bees used in our studies was made by Mr. C. E. Atwood. In this he was greatly assisted by material of *Halicti* determined by Miss Grace Sandhouse, Bureau of Entomology, Washington, and of *Andrenae* by Dr. T. D. A. Cockerell, Boulder, Colorado.

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VARIATION IN *CLOSTRIDIUM WELCHII*[1]

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Abstract

In this paper it is shown that by plating cultures of *Cl. welchii*, on a suitable hormone-blood agar, two distinct colony types, regarded as S and R, and frequently intermediate types, may be isolated. Once isolated and stabilized by continued cultivation and selection at least the two more extreme types show true breeding properties. Serial cultivation in various fluid media, particularly Robertson's chopped meat, has resulted in both S to R and R to S variation. This has been observed in strains purified by colony selection and by single cell isolation.

The S type has proved to be highly virulent for pigeons, the R type much less virulent but there is some evidence that the apparent killing action of R types may result from *in vivo* dissociation of the R to S. Both S and R types are shown to yield hemotoxin which is neutralized by antitoxin but under similar conditions S types produce 10 to 20 times more toxin than R types.

Introduction

In recent work on the *in vitro* and *in vivo* action of *Cl. welchii* (Reed, Orr and Burleigh (7) and Orr, Campbell and Reed (5)) much difficulty was experienced owing to variation in the pathogenicity of the same strain of organisms at different times and in the potency of different lots of toxin apparently prepared in the same manner. Two possible explanations suggest themselves: either, variation of the organisms or difference in toxin production as a result of possible differences in successive lots of the culture media. The influence of the medium will be discussed in a subsequent paper.

In a preliminary paper (6), it was shown that *Cl. welchii* displays variation or dissociation to a conspicuous degree and that by a process of selection at least two variant types may be isolated from pure cultures, types which differ in colony structure, pathogenicity and toxin production. In the present paper more detailed evidence of this variation is presented. The only instances of this sort of variation among the anaërobic bacteria which appear to have been reported are short notes by Sordelli and Ferrari (9) on *Cl. septique*, and Spray (10) on several species. Personal communications from Professors Hadley of Michigan and Maitland of Manchester indicate that they have made similar observations.

Experimental Methods

The several cultures of *Cl. welchii* used in this investigation had been carried in stock for a number of generations, in a chopped-beef infusion broth medium. Dr. I. C. Hall of the University of Colorado very kindly sent to us two further strains designated C_4 and C_5.

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In the study of variation based initially on colony structure, cultures were made on plates of a hormone agar prepared essentially as described by Huntoon (1) enriched with 0.1% of glucose and 5% of defibrinated rabbit blood. The plates were incubated at 37.5° C. in Fildes jars; phosphorus jars, as modified by Varney (11); or in pyrogallol jars. The former, especially when calcium chloride was used in the bottom of the jar in order to ensure that the medium had a relatively dry surface, proved most convenient although equally good results were obtained by the three methods. An incubation period of 48 hr. was found to give the most distinctive colony forms. The inoculum was always very light in order to ensure the development of discrete colonies; best results were obtained with plates showing not more than 100 to 200 colonies. On this medium and under these conditions, growth of *Cl. welchii* was much more luxuriant and colonies were much larger than those which have generally been described. When plates of this medium and blood medium made with ordinary beef-extract agar were inoculated from the same emulsion, the former yielded colonies several times larger than the latter even when the two were incubated in the same anaërobic jar. Moreover, where variation occurred the differences in colony form were very much more conspicuous on the hormone than on the ordinary blood agar.

Two Colony Forms

By the procedure outlined it was possible to isolate at least two types of colonies from all of the cultures studied. One, considered the S type, resembles the colony form usually described as characteristic of *Cl. welchii* (4, 8, 12, 14). This is a smooth regular low mound, sometimes slightly umbonate, with a circular outline and entire margin greyish-white in color with a smooth glistening surface (Fig. 1). These colonies showed considerable variation in size, ranging from two millimetres or less up to five or six millimetres in diameter, depending upon the degree of crowding on the plate.

A second distinct colony type, the R, is strikingly different. This is a large, spreading, perfectly flat colony, frequently reaching a diameter of a centimetre and a half with a very irregular flagellated margin and a finely granular dull surface (Figs. 3 and 4). At times the outline of the colonies may become extremely irregular with feathery prolongations but with the same granular surface (Fig. 5). Occasionally the central part of the colony shows a slightly elevated area but usually this is lacking. These colonies look more like the figures of *Cl. sporogenes* and *Cl. oedematiens* (4) than classic *Cl. welchii* colonies.

In addition to these two extreme colony types, plate cultures, especially those made from old broth cultures, often exhibit a number of apparently intermediate forms. In cultures predominantly S in form colonies frequently appear with slightly serrate margins and a somewhat irregular surface, while in cultures predominantly R there may appear colonies with a smooth dome-shaped central area but with characteristic spreading, granular margins. Still other cultures produce colonies which are so nearly intermediate between

the two extreme types that classification is impossible. Subcultures of these intermediate forms generally developed S, R or mixtures of S, R and intermediate types.

In a few instances mucoid modifications of both the S and R colony forms were observed. These colonies are indistinguishable in appearance from the typical S and R forms. While the ordinary or non-mucoid S colony consists of a soft gelatinous mass readily emulsified in saline or water to form an even and highly stable suspension, the similar-appearing mucoid S colony when touched with a loop may be drawn out into mucilaginous threads one to five centimetres long. The non-mucoid R colonies though soft and gelatinous are rather more granular than the S, and emulsions, as noted in more detail later, are highly unstable. The mucoid R colonies when touched with a loop behave in the same manner as the mucoid S colonies.

Deep Colonies

Colonies of S and R types developing below the surface of hormone agar, as in poured plates or shake agar tubes, show such differences as might be anticipated from the topography of the surface colonies. The typical S colony growing under such circumstances is a lense-shaped structure of pale milky opalescence with sharply defined margins and resembles the deep colonies of *Cl. welchii* described by Weinberg and Séguin (12). In contrast, the typical R growing as a well-isolated submerged colony appears as a loose filamentous or woolly mass.

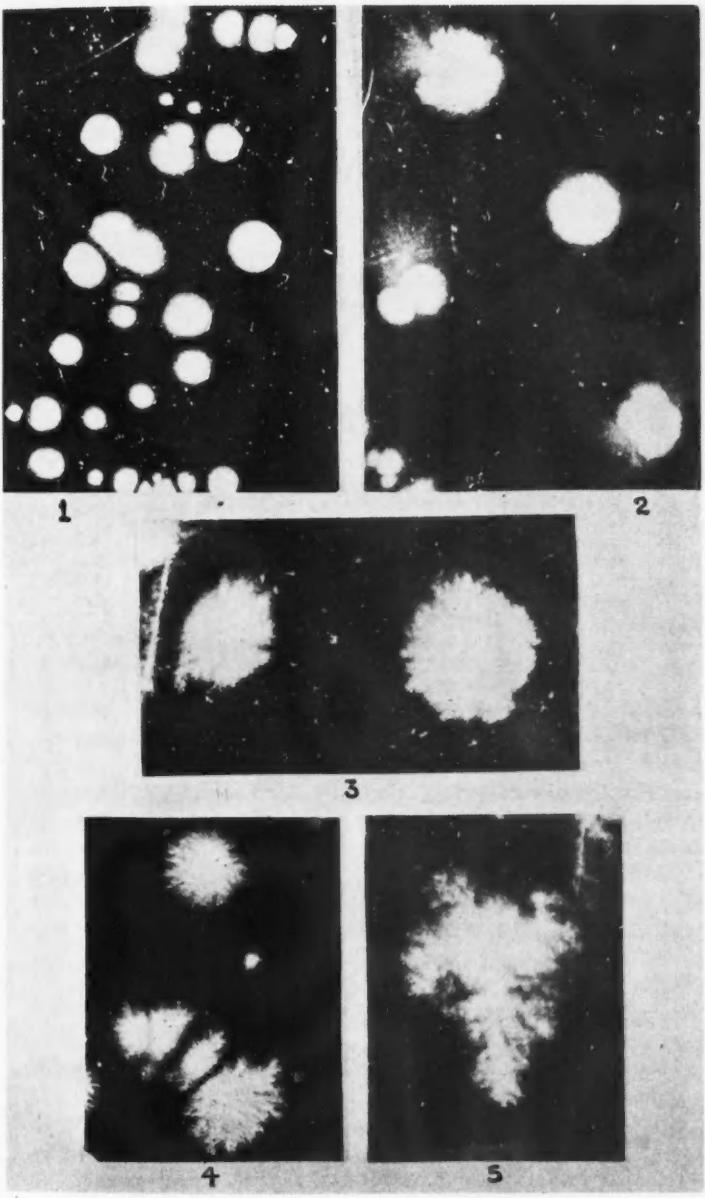
Morphology and Capsulation

The individual organisms of the S type are characteristic of *Cl. welchii* as ordinarily described, rather short, plump bacilli with rounded ends, generally occurring singly or occasionally in chains of two or three end to end. Staining is uniform with the ordinary dyes and definitely positive with Gram's procedure. From most culture media the organisms show capsules one to two times the thickness of the body of the bacilli. Organisms of the R type, in contrast, are much more irregular in form; generally, in films from fluid cultures, the majority are short plump bacilli like the S type, but short coccoid forms and many long bacilli, 5 to 20 μ, are to be found. Chain formation, especially in the case of the long forms, is much more common than in the case of the S type. While the S stain uniformly the R types show much granulation; with methylene blue or even with Gram's stain most organisms in a film appear to consist of a mass of fine heavily staining granules contained in an unstained matrix. Capsules, so characteristic of the S type, are almost completely absent in the R type.

Habit of Growth in Fluids, and Cataphoresis

Well-differentiated cultures of the S type always produce in fluid media a diffuse growth in which the organisms remain uniformly suspended for long periods. Definite R cultures on the other hand produce first a diffuse followed by a bottom growth which leaves the supernatant fluid perfectly clear. It

PLATE I



Photographs of colonies of Cl. welchii growing on hormone-blood agar. FIG. 1. *S colonies.* FIG. 2. *S colonies with very thin R outgrowths.* FIGS. 3 AND 4. *R colonies.* FIG. 5. *Widely spreading R colony. The photographs were made by B. Halsgrove.*

has generally been found that if serial fluid cultures of an S type begin to develop bottom growth, plates will show R colonies. On the other hand when serial cultures of an R type show persistent diffuse growth in the supernatant fluid, plates will indicate the presence of S types.

This habit of growth appears to be associated with the surface charge on the organisms. As indicated in Table I, when organisms of well-differentiated S and R types were washed in the centrifuge three times and suspended in Clark's phosphate buffer solutions the R suspensions proved highly unstable, the S suspensions relatively stable: the R organisms agglutinated

TABLE I

ACID AGGLUTINATION OF S AND R *Cl. welchii*

pH	R from C_4	S from C_4	R from C_8	S from C_8	pH	R from C_4	S from C_4	R from C_8	S from C_8
1.2	+	−	+	−	4.8	+	+	+	+
1.4	+	−	+	−	5.0	+	+	+	+
1.6	+	−	+	−	5.2	+	+	+	+
1.8	+	+	+	−	5.4	+	+	+	+
2.0	+	+	+	+	5.6	+	−	+	
2.2	+	+	+	+	5.8	+	−	+	−
2.4	+	+	+	+	6.0	+	−	+	−
2.6	+	+	+	+	6.4	+	−	+	−
4.4	+	+	+	+	6.8	−	−	+	−
4.6	+	+	+	+	7.2	−	−	+	−

NOTE:—*Washed organisms of the two types were suspended in phosphate buffer solutions; + indicates complete agglutination and precipitation, and − indicates no agglutination.*

over the entire pH range tested from 2.0 to 6.4 or 7.2; the S organisms were agglutinated in mixtures with a pH range of 1.2 to 5.4 or 5.6 but remained in stable suspension in the more acid and basic mixtures. Similar suspensions tested in the Kunitz type cataphoresis apparatus exhibited isoelectric points in agreement with the acid agglutination results. Suspensions of well-established S types from various cultures were isoelectric in buffer mixtures from pH 2.0 to 2.4 with an average of pH 2.1. Suspensions of well-established R types from various cultures were isoelectric from pH 3.4 to 3.8 with an average at pH 3.6 and types showing intermediate colony forms generally exhibited intermediate isoelectric points.*

Origin of the Two Colony Types

The two colony types were most readily obtained by plating, as just described, from old broth or chopped-meat media cultures. On plating such old cultures for the first time usually a majority of S colonies developed but at the same time, in most instances, either a few or many flat irregular colonies of the R type also appeared. Frequently too these primary plates developed S colonies with marginal outgrowths of R-like structure, as indicated in

**More data on the relation of cataphoresis to the variation of this species will be presented in collaboration with B. G. Gardiner.*

Fig. 2. Where well-isolated and characteristic S and R colonies appeared on primary plates, pure cultures of the R form were generally obtained on the first subculture from R colonies. The R-like marginal outgrowths from S colonies likewise frequently produced pure growths of the R type though usually the first culture consisted of R and S types, and several selections were necessary before pure R cultures were obtained. In either case when this type was finally purified it exhibited a high degree of stability; after many cultural generations on this solid medium there was no apparent tendency to variation.

The S forms on the other hand, as in the case of most other species of bacteria studied from this point of view, proved to be less stable. The selection and subculture of S types in many instances resulted in the development of pure S cultures which have bred true for many generations on this solid medium. Frequently the S cultures grown from single S colony selections have continued even after many generations to produce a small proportion of S colonies with R-like outgrowths. Continued single colony selection has, however, always resulted in the development of true breeding and relatively stable S types, as long as the culture was maintained on this solid medium and propagated from single characteristic colonies.

More precise evidence of the origin of one variant type from the other has been obtained by growing carefully purified types in fluid media. In one instance a culture of strain C_4, which for eight generations on solid media had exhibited only S types, was introduced into various fluid media (listed in Table II) and transferred serially at 24-hr. intervals. Under these circum-

TABLE II

S TO R VARIATION IN *Cl. welchii*, C_4

Fluid media	Colony form
9 generations in ordinary broth with 0.1% glucose	All S
4 generations in hormone broth with 0.1% glucose	All S
10 generations in hormone broth without glucose	All S
30 generations in chopped meat with 0.1% glucose	Mostly S, few R
1 generation in chopped meat with 0.1% glucose aged 8 weeks at room temperature	Mostly S, few R

NOTE:—*One characteristic S colony from the eighth generation on solid media showing only S colonies was used to inoculate the fluids. Variation was determined by plating on hormone-glucose-blood agar.*

stances, as indicated in Table I, the S type exhibited a high degree of stability although plates made from the 30th serial culture in a chopped-meat medium developed a few R colonies. Plates from an eight-weeks-old culture in a similar medium likewise developed a few R colonies. In both cases subcultures of the R colonies resulted in true breeding R types.

The possibility of R to S variation may be illustrated by one series in which an R type of strain C_4, after producing only R colony types for eight generations on solid media, was transferred to various fluid media (listed in

Table III). On plates made from the fourth serial 24-hr. culture in ordinary broth containing 0.1% of glucose, the growth consisted of 80% R and 20% S colonies. Subcultures of several R colonies developed only R types. Sub-

TABLE III

R TO S VARIATION IN *Cl. welchii*, C_4

Fluid media	Colony form
4 generations in ordinary broth with 1% of glucose	80% R, 20% S
12 generations in hormone broth without glucose	All R
12 generations in hormone broth with 1% of glucose	All R
6 generations in chopped meat with 1% of glucose	All R

NOTE:—*One characteristic R colony from the ninth generation on solid media showing only R colonies was used to inoculate the fluids. Variation was determined by plating on hormone-glucose agar.*

cultures of several characteristic S colonies developed a mixture of R and S types but after two additional selections of the most characteristic S colonies a true breeding S was obtained from this strain, originally R.

Single Cell Isolations

Results obtained with cultures purified by colony isolations were confirmed with cultures purified by single cell isolations. The methods of isolation used by Kahn (2) and by Wright and McCoy (13) were employed and the selected single cells were planted in tubes of recently boiled Robertson's chopped meat by the procedure of breaking the tips of the micropipettes below the surface of the fluid. Wright and McCoy (13) make the general statement that, in working with anaërobes, about 2% of the single cells isolated may be expected to develop. In one series with *Cl. welchii* where 50 cells were isolated from five different cultures, three (6%) grew into characteristic cultures.

An S type of C_4 strain which had been purified by repeated single colony isolation and which had produced only S colonies for eight successive platings on the hormone-blood agar was transferred to Robertson's chopped meat. After 24 hr. growth several single cells were isolated and placed in similar chopped-meat tubes. Three cultures developed.

These three cultures were plated in the usual manner, as just described, after 24 hr. growth in the Robertson's meat from the single cell inoculations. From the first two cultures, plates containing several hundred colonies exhibited only characteristic S forms like the parent culture. Plates from the third single cell culture developed, in addition to several hundred characteristic S colonies, two colonies showing minute R-like marginal outgrowths similar to those pictured in Fig. 3. Subcultures from these R-like outgrowths on solid media developed R and S colonies and by further colony selection pure R type cultures were obtained. It seems apparent therefore that one cultural generation in Robertson's meat from a single cell isolation may result in S to R variation.

The two remaining cultures developed from single cells were transferred to the fluid media listed in Table IV. As noted in the table, except for the series in ordinary glucose broth, all showed some S to R variation. Essentially the

TABLE IV

S TO R VARIATION IN A CULTURE PURIFIED BY SINGLE CELL ISOLATION

Fluid media	Colony form
10 generations in ordinary broth with 0.1% of glucose	All S
8 generations in chopped meat with 0.1% of glucose in an atmosphere of 10% CO_2	Mostly S, very few with R outgrowths
11 generations in chopped meat with 0.1% of glucose, 40° C.	Mostly S, few with R margins
23 generations in chopped meat with 0.1% of glucose, 37° C.	Majority S, many intermediate, many characteristic R
1 generation in chopped meat with 0.1% of glucose, 24 hr. at 37° and 2 months at 12 to 15° C.	Majority S, many intermediate, many characteristic R

NOTE:—*The media were inoculated from the first generation in chopped meat from a single-cell isolation. Variation was determined by plating on hormone-glucose-blood agar.*

same results were obtained with the two cultures. In both instances, by colony selection and subculturing pure R-type cultures were obtained from the original S cultures.

It is apparent therefore that similar degrees of variation of S-type organisms have been obtained from cultures purified by colony selection and by single cell isolation.

Pathogenicity

Pathogenicity has been determined by injecting the fluid portion of Robertson's chopped-meat cultures, after 24 hr. growth, into the breast muscle of pigeons. Both the S and the R types prove to be pathogenic but the minimum killing dose of the R type is in general approximately 10 times greater than the minimum dose of S where the two types have been isolated from the same strain. Results from such tests, with a culture of relatively low pathogenicity, are indicated in Table V. In this instance 5 cc. of the R type produced approximately the same reaction as 0.5 cc. of the S type. Similar proportionate killing effects with S and R cultures from other strains have been observed.

TABLE V

PATHOGENICITY OF S AND R TYPES OF *Cl. welchii* FOR PIGEONS, AS INDICATED BY THE KILLING ACTION OF 24-HR. CULTURES

Type	Dose, cc.	Effect
S type C_4	0.1	Toxic, but birds recovered
	0.5	Death in 12 to 16 hr.; small amount of edema
	1.0	Death in 8 to 10 hr.; marked edema and necrosis
R type C_4	1.0	Death in 7 to 10 days; marked edema and necrosis
	5.0	Death in 12 to 16 hr.; small amount of edema

There is now, however, considerable evidence to indicate that there is a fallacy in this method of distinguishing between the pathogenicity of the S and R types. In most instances where pigeons have been infected intramuscularly with R types and the edematous or necrotic area has been cultured after the death of the bird, R types similar to those injected, together with an appreciable percentage of S types (in some cases as high as 50% of S types), have been recovered. This has been most frequently observed where a relatively small dose of the R culture has been used so that the animals survived for several days. Since these S types are invariably highly virulent it is impossible to suppose that they were previously present in the tissue. This suggests, obviously, that the death of the animals may result from the S types arising from an R to S dissociation *in vivo*.

Hemotoxin Production

Hemotoxin production was determined by the methods used in earlier work in this laboratory with *Cl. welchii* toxin (5, 7). The organisms were grown in the usual chopped-beef–peptone medium enriched with 0.1% of glucose as recommended by Bengston and incubated for 24 hr., the supernatant material centrifuged, filtered and the filtrate titrated immediately. The titration consisted in determining the concentration of the toxic filtrate which would produce hemolysis of a 2% suspension of washed rabbit red cells.

Toxins were prepared in this way from well-established S and R types and from the undissociated parent strains from which the dissociated types were obtained. One such titration is shown in Table VI. Both R and S

TABLE VI

TITRATION OF HEMOTOXIN PRODUCED FROM S AND R TYPES AND FROM THE PARENT STRAIN OF A CULTURE OF *Cl. welchii* WITH AND WITHOUT ANTITOXIN

	Dilutions of toxin								
	$\frac{1}{5}$	$\frac{1}{10}$	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$	$\frac{1}{320}$	$\frac{1}{640}$	Cont.
S type, C_4	+	+	+	+	+	+	+	−	−
S type, C_4, plus antitoxin	−	−	−	−	−	−	−	−	−
R type, C_4	+	−	−	−	−	−	−	−	−
R type, C_4, plus antitoxin	−	−	−	−	−	−	−	−	−
C_4, original culture	+	+	+	−	−	−	−	−	−
C_4, original culture with antitoxin	−	−	−	−	−	−	−	−	−

types, it may be noted, produced hemotoxin but in this instance the S produced 20 times more toxin than the R and about six times more than the undissociated parent culture. Results were similar with other strains and in many preparations from the same strain.

The hemotoxin produced by both the S and R organisms was neutralized by a stock *Cl. welchii* antitoxin. In the titration results shown in Table VI a 1 to 100 dilution of a stock *Cl. welchii* horse-serum antitoxin was mixed

with the various dilutions of the toxin and incubated for half an hour before the addition of the rabbit red cells. This dilution of the antitoxin completely inhibited both the S and the R hemotoxin.

In vivo Action of Hemotoxin

Much earlier work has indicated that the intravenous introduction of suitable doses of *Cl. welchii* toxin into rabbits produces a profound anemia accompanied by cellular changes which resembles pernicious anemia in man (5, 7). A particular sample of toxin recovered from an S type of culture C_4 in 2.5-cc. amounts produced such a change in rabbits, but a similar amount of filtrate from an R type recovered from the same culture produced negligible blood changes. The quantitative changes in red cells and hemoglobin produced by the same amounts of filtrates from S and R types and from the undissociated parent culture are shown in Table VII.

TABLE VII

THE *in vivo* ACTION OF S AND R TYPES AND THE UNDISSOCIATED PARENT CULTURE OF *Cl. welchii*

Toxin	Drop in red cells, %	Drop in hemoglobin, %
S type, 2.5 cc.	66	70
R type, 2.5 cc.	9	8.5
Undissociated, 2.5 cc.	25	52

TABLE VIII

In vivo ACTION OF EQUAL HEMOLYTIC DOSES OF S AND R *Cl. welchii* TOXIN IN RABBITS

Toxin	Drop in red cells, %	Drop in hemoglobin, %
C_4S, 2.5 cc.	66	70
C_4R, 2.5 cc.	43	38

The *in vitro* action of this sample of S hemotoxin was approximately 10 times greater than the R toxin. By taking this hemolytic titre as the criterion, equal hemolytic doses of toxin were introduced intravenously into rabbits. One such result is shown in Table VIII where it is apparent that even when equal hemolytic doses are introduced, the S type toxin produces a more profound anemia than the R type.

Antigenic Activity

Immunization of rabbits with repeated doses of heat-killed S type whole organisms results in sera which agglutinate homologous S organisms but not R organisms from the same culture. This phase of the problem will be reported in more detail in a subsequent paper.

Species Characteristics of the Two Types

Both the S and R types behave as characteristic *Cl. welchii*. Both types produce a similar degree of stormy fermentation in milk and both produce similar fermentation reactions in those carbohydrates which have ordinarily been used in the differentiation of anaërobic bacteria. Results of such carbohydrate fermentations with S and R types isolated from three strains of *Cl. welchii* are shown in Table IX. It is apparent from the table that on the

TABLE IX

CARBOHYDRATE FERMENTATIONS PRODUCED BY S AND R TYPES OF THREE STRAINS OF *Cl. welchii*

Type and strain	Milk	Glucose	Lactose	Saccharose	Galactose	Levulose	Maltose	Glycerol	Mannite	Inulin	Salicin	Dulcite
S type, C_4	Stormy	+	+	+	+	+	+	−	−	−	−	−
R type, C_4	Stormy	+	+	+	+	+	+	−	−	−	−	−
S type, C_5	Stormy	+	+	+	+	+	+	−	−	−	−	−
R type, C_5	Stormy	+	+	+	+	+	+	−	−	−	−	−
S type, L	Stormy	+	+	+	+	+	+	−	−	−	−	−
R type, L	Stormy	+	+	+	+	+	+	−	−	−	−	−

basis of glycerol and inulin fermentation the three strains belong to *Cl. welchii* type IV, but more significant from the point of view of this investigation was the fact that the R and the S types give the same fermentations.

It is evident from these results that notwithstanding the marked differences in colony topography, pathogenicity and antigenic structure that the enzyme contents of the two types, as far as tested by these reactions, are the same.

Note

After this paper was completed the work of C. A. McGaughey (3) came to the writers' attention. Using a somewhat similar procedure to that just described McGaughey obtained from a culture of *Cl. welchii*, in addition to what are described as normal colony forms (apparently the same as the writers' S colonies), two variants described as the variant 1 and variant 2. Variant 1, in colony structure pathogenicity and hemotoxin production, appears to be the same as the colony form which the present writers have called R. Variant 2 resembles less closely what has been described herein as types intermediate between S and R. Mucoid variants of the normal form and variant 1, which McGaughey describes, resemble the authors' mucoid forms of S and R. It is evident, therefore, that although McGaughey's observations and the writers' are not in complete agreement the results support each other in indicating that *Cl. welchii* is subject to profound variation.

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THE RATE OF DECOMPOSITION OF CREATINE IN ACID AND IN ALKALINE SOLUTION[1]

By Alexander Thomas Cameron[2] and Jean Stirton Guthrie

Abstract

Studies have been made on the decomposition of creatine in acid and alkaline solutions at 37.5° and 50° C. The rates of transformation to creatinine in acid solutions gave values for the Arrhenius constant varying from 20,700 to 24,000, values in moderate agreement with those of Edgar and Wakefield.

There was a definite minimal rate of transformation at approximately 0.1 *N* hydrochloric acid, and a secondary maximum at about 0.01 *N* hydrochloric acid. This was probably owing to catalysis of creatine hydrochloride and free creatine at different rates. The slightly differing values found for the Arrhenius constant in strong and in weak acid solutions may be explicable on this basis.

The results with alkali support Gaebler's views that creatine is initially transformed into urea and sarcosine, which then reunite to form ammonia and methyl hydantoic acid.

These results show further that in solutions of acid and alkali of corresponding strength, alkali catalyses the change from creatine to creatinine much more rapidly than acid.

It appeared from a recent survey of the biochemical application of the Arrhenius equation for the effect of temperature upon rate of reaction (4) that further determination of the Arrhenius constant for reactions *in vitro* that are of biochemical significance might be of some value. The transformation of creatine into creatinine was selected for initial consideration since numerous studies of this change have been recorded and the constant has already been determined by Edgar and Wakefield (7). Hence study of this change seemed very suitable for the purpose of checking accuracy of technique. The results, while largely confirming those of Edgar and Wakefield, disclosed certain facts which do not appear to have been hitherto recorded and seem to be of sufficient significance to be worth reporting. The results also lend support to Gaebler's views on the decomposition of creatine in alkaline solution.

Experimental Technique

A preliminary series of measurements was made with 0.5% commercial creatine hydrate and different strengths of hydrochloric acid and sodium hydroxide, at temperatures of 37.5° and 50° C. The concentration of creatine was selected as being almost the strongest possible for the method employed, and as corresponding roughly to the average creatine content of striped muscle. These preliminary measurements were of great value in indicating approximate results, since they permitted, in the subsequent accurate series, colorimetric comparison between alkaline creatinine picrate solutions of almost equal strengths.

For the accurate series a good commercial creatine was recrystallized once from hot water. Attempts to air-dry this to give the pure hydrate failed

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since, in midwinter in Winnipeg, air-drying caused a slow loss of water from the hydrate. Hence the preparation was dried to constant weight (six days) at 105° C. It analyzed (mean of four determinations) 32.04% nitrogen (by Kjeldahl) as compared with the theoretical value 32.06%. A test in which 0.44 gm. was extracted with *cold* water at room temperature showed, by colorimetric procedure, no detectable trace of creatinine.

In the preliminary series commercial creatinine was used for colorimetric comparison. For the accurate series a good commercial creatinine zinc chloride was recrystallized from hot water and air-dried for three days. It then contained 0.3% of water; further air-drying did not alter this content. It was therefore used and corrected for this trace of moisture, 1.608 gm. per litre being taken as equal to 0.1% of creatinine. It analyzed (corrected for water content) 22.80% nitrogen (mean of three determinations; theory 23.18%) and 19.35% chlorine (mean of two determinations; theory 19.57%).

Twice-distilled water was employed for all creatine and creatinine solutions and for dilution of all acid and alkaline solutions. Mallinckrodt c.p. hydrochloric acid and Kahlbaum (alcohol-purified) sodium hydroxide were used, the oxalic acid used for standardization was a B.D.H. A.R. preparation. Titration values for acid and alkaline solutions indicated an accuracy almost always within 0.2%.

In the accurate series the temperatures of the water bath were 37.5° and 50.2° C., both $\pm 0.1°$ and usually $\pm 0.05°$ (standardized thermometers). Standardized pipettes, burettes, and flasks were invariably employed.

For each experiment an accurately weighed amount of creatine was dissolved in water rapidly with application of slight heat. Equal volumes of the solution and of acid or alkali of suitable strength were pipetted, one into a wide-mouthed 500-cc. bottle, and another into a tall weighing bottle introduced within it. The large bottle was then closed with a rubber cork and almost completely immersed in the thermostat. When its contents had attained the temperature of the bath (30 or 40 min.) the weighing bottle was overturned with a glass rod, immediate shaking producing an accurate zero time. The mixed solution of course contained creatine and acid or alkali of strength half that prior to mixing. At suitable time intervals an exact amount of solution was pipetted into an equal volume of alkali or acid of strength corresponding to that of the mixed solution, thus producing immediate neutralization and stopping the reaction. Five cubic centimetres was usually used, 2 cc. when the amount of creatinine was large.

Estimation of Creatinine

The usual Folin colorimetric procedure was slightly modified. To the 10-cc. neutralized sample was added 10 cc. of alkaline picrate. The approximately correct amount of suitable standard was measured from a burette and distilled water added from a second to total 10 cc., and to this 10 cc. of alkaline picrate was also added. After five minutes comparisons were made rapidly in a micro-colorimeter, using artificial illumination.

Two standards were employed, one containing 0.1% creatinine, the other 0.02%. In estimating very dilute solutions of creatinine (less than 0.4 mgm. of creatinine in the 5-cc. sample) it was found necessary to add a measured amount of standard to the unknown. The subsequent correction of course increased the possible error. The lengths of columns of standard were adjusted to suitable depth of color. Solutions containing more than 3 mgm. of creatinine in the original 5-cc. sample required further dilution with water to an extent depending on depth of color. Throughout, parallel dilution procedures were used with unknown and standard.

The alkaline picrate was made up daily in the ratio of 100 cc. of saturated solution of picric acid to 20 cc. of exactly 10% sodium hydroxide. Tests showed that such alkaline solutions 18 hr. old gave perfect correspondence with those freshly prepared. The picric acid used was purified by the procedure of Halverson and Bergeim (cf. Hawk and Bergeim (12)).

In making the actual colorimetric reading the standard was placed in both cups, the light incidence and the cup level on one side adjusted to perfect color match, and the solution in that cup replaced by the unknown and four readings taken, the mean of these being used.

Factors affecting the probable error of the creatinine estimation. Difference in degree of alkalinity between standard and unknown picrates to the extent of 0.01 *N* gave no error, to the extent of 0.025 *N*, no definite error, but to the extent of 0.05 *N* gave a marked difference (about 17%). Addition of sodium chloride to give a concentration of 1.8% did not affect the standard. This was equivalent to the amount formed in neutralizing the stronger acid and alkaline solutions.

Comparison of two known solutions of relative strengths 4 : 5 gave results differing by 0.5% or less from accuracy. Few of the comparisons made in the accurate series exceeded this ratio.

Two important sources of error were found. The creatinine zinc chloride standards employed definitely deteriorated with time. The error for the stronger standard was negligible for three weeks, but thereafter the strength decreased with increasing rapidity. The weaker standard deteriorated slightly more rapidly. In a few of the earlier experiments corrections had to be applied for this error.

Application of heat to bring creatine into solution involves a slight conversion to creatinine, the amount formed varying with the temperature. Hence in every case a zero determination was necessary in the creatine solution. The usual correction was found to be about 2 mgm. per 100 cc.; obviously where the rate of change is small the exactitude of this correction is important. In aqueous solutions of creatine that were being heated in the water bath to the experimental temperature (before mixing with acid or alkali) the error due to this application of heat was not measurable at 37.5° but was just definite at 50° C. Hence for experiments at the latter temperature the zero correction was determined by immersing the creatine solution at 50° C. for 40 min. before estimation.

It is impossible to determine the probable error of the creatinine determinations by statistical methods, since it varied chiefly with the degree of fatigue of the eye. Slight fatigue appeared to be desirable, apparently due to an enhancement effect (cf. Allen, 1, 2, 3). Experience showed that only one accurate set of readings of the reddish-orange colors could be made in each half hour, and after several hours of such half-hourly readings fatigue effect was evident through decreasing agreement in consecutive readings. Moreover, experiments over several consecutive days showed decreasing accuracy from day to day.

Error from unequal illumination is marked but avoidable.

In spite of the large potential fatigue error the agreement that was actually obtained for like conditions in different experiments suggests that the mean of three experiments was almost always within 2% of accuracy, if not within 1%.

Estimation of total creatinine. In the few experiments (with alkaline solutions) in which such estimations were made, since large amounts had to be measured, 5-cc. samples were taken, exactly neutralized with 5 cc. of acid, then 10 cc. of 2 *N* hydrochloric acid added and the mixture autoclaved for 30 min. at 18 to 20 lb. pressure. After cooling 10 cc. of 2 *N* sodium hydroxide and 25 cc. of alkaline picrate were added, and after five minutes the whole was diluted to 250 cc. and compared with a standard of similar strength of creatinine.

Estimation of urea in alkaline creatine solutions. The usual sampling procedure was employed, but since each sampling permitted loss of ammonia from the solutions duplicate 10-cc. samples were removed at each period, and immediately neutralized. The ammonia evolved from one sample, after addition of alkali, was carried over with a stream of air into *N*/50 acid for pre-formed ammonia, and the other was decomposed with urease and then similarly treated, the difference giving the true urea value. An error of one drop of *N*/50 alkali in the final titration corresponded to 0.03 mgm. of urea or 0.07 mgm. of creatine per 100 cc. of solution. Subtraction of the ammonia value doubles the possible error from this source.

Estimation of ammonia in alkaline creatine solutions. Since much of the ammonia passes to the gas phase consecutive sampling could not be employed. Immediately after mixing creatine and alkali, 10-cc. samples were transferred to each of eight thick-walled urea tubes already at the experimental temperature and these were tightly stoppered. One was used for a zero determination, the others removed at definite time intervals, the ammonia aerated into *N*/50 acid and this titrated against alkali. The error was of an order corresponding to that for urea.

Experimental Results

Table I illustrates the degree of agreement (and non-agreement) in the results of a single series. The figures give the number of mgm. of creatinine produced in 100 cc. of a solution of 0.4396% creatine (equal to 0.5% creatine hydrate) and 0.1 *N* hydrochloric acid at 37.5° C.

TABLE I

A TYPICAL SERIES

Time, hr.	Experiment No.								Mean
	1	2	3	4	5	6	7	8	
0.5	4.6	4.5	—	—	4.6	—	—	—	4.6
1.0	—	8.9	8.5	8.6	—	—	—	—	8.7
1.5	—	—	—	12.2	12.4	—	—	12.8	12.5
2.0	15.2	15.7	—	—	—	15.5	—	—	15.5
2.5	—	—	—	—	—	18.7	18.6	18.8	18.7
3.0	—	—	—	—	22.7	22.3	—	22.9	22.6
3.5	—	—	—	27.4	—	28.8	—	28.2	28.1
4.0	—	33.4	32.6	32.8	—	—	—	—	32.9
5.0	—	—	—	—	—	40.8	40.9	41.2	41.0
6.0	—	48.9	49.3	—	—	48.5	—	—	48.9
7.0	—	—	55.7	—	—	—	—	—	(55.7)
8.0	—	63.1	—	—	—	—	—	—	(63.1)

NOTE:—*Creatine, 0.4396%; hydrochloric acid, N/1; temp., 37.5° C.*

The results for different strengths of acid at 37.5° are shown in Table II, and at 50.2° C. in Table III, the concentration of creatine being always 0.4396%. Table IV presents results in acid solution for different creatine concentrations. All figures are in mgm. of creatinine per 100 cc. of solution. Those asterisked are for single determinations; the others are almost all the means of three determinations.

Table V gives the results for creatinine, total creatinine, urea, and ammonia in solutions containing 0.4396% of creatine and 0.1 *N* sodium hydroxide at 50.2° C., all figures being in mgm. per 100 cc.

TABLE II

CREATININE PRODUCED IN ACID SOLUTION

Time, hr.	Normality of hydrochloric acid								
	2.0	1.0	0.33	0.10	0.033	0.010	0.0033	0.0010	0.0001
0.5	12.4	4.6	—	—	—	—	—	—	—
1.0	22.4	8.7	2.2*	1.1*	2.1*	4.1	4.4*	1.7*	—
1.5	28.7	12.5	—	—	—	—	—	—	—
2.0	40.2	15.5	4.4*	1.9*	—	8.5	6.8*	—	—
2.5	50.8	18.7	—	—	—	—	—	—	—
3.0	59.0*	22.6	—	—	6.2*	12.6	—	5.1*	—
3.5	65.3	28.1	—	—	—	—	—	—	—
4.0	—	32.9	8.8*	—	—	16.3	10.8*	—	—
4.5	—	—	—	3.0*	—	—	—	—	—
5.0	87.3	41.0	9.5*	—	8.2*	18.9	—	7.3*	—
5.5	—	—	—	—	—	—	—	—	1.3*
6.0	99.9	48.9	12.2*	3.9*	—	22.6	17.4*	—	—
7.0	—	55.7*	—	—	—	24.5*	—	9.4*	—
8.0	—	63.1*	—	4.1*	—	—	—	—	—
24.0	—	—	—	13.1*	—	79.0*	—	19.6*	—

NOTE:—*Creatine, 0.4396%; temp., 37.5° C.*

TABLE III

CREATININE PRODUCED IN ACID SOLUTION

Time, hr.	Normality of hydrochloric acid					
	2.0	1.0	0.33	0.10	0.033	0.010
0.25	21.7	9.6	—	—	—	—
0.50	44.1	17.6	—	—	—	—
0.75	66.3	27.6	—	—	—	—
1.00	86.8	32.8	7.4*	1.5*	5.1*	15.1
1.25	116.8	45.9	—	—	—	—
1.50	—	52.6	—	—	—	—
2.0	161.4	66.8	15.4*	—	—	29.8
2.5	—	—	—	—	—	37.4*
3.0	233.7	92.2	—	6.1*	—	44.3
3.5	—	—	—	—	—	50.5
4.0	—	119.6	—	—	22.5*	57.4
5.0	—	147.2	—	—	—	66.9
6.0	—	—	—	—	—	81.0
7.0	—	—	—	15.4*	—	92.7
8.0	—	—	—	—	—	115.8

NOTE:—*Creatine, 0.4396%; temp., 50.2° C.*

TABLE IV

RATE OF CREATININE PRODUCTION FOR VARYING CONCENTRATION OF CREATINE

Conc. creatine, %	Normality HCl	Time, hr.		
		1	2	3
0.4396	1.0	32.8	66.8	92.2
0.2198	1.0	14.7*	28.5*	42.0*
0.1099	1.0	8.4*	14.4*	20.9*
0.4396	0.01	15.1	29.8	44.3
0.2198	0.01	6.5*	12.6*	20.0*
0.1099	0.01	2.6*	5.3*	7.3*

NOTE:—*Temp., 50.2° C.*

TABLE V

ESTIMATION OF DECOMPOSITION PRODUCTS OF CREATINE IN ALKALINE SOLUTION

Time, hr.	Creatinine	Total creatinine	Urea	NH_3
0.5	118	353*	—	—
1.0	137	346*	8.0*	0.9
1.5	144	—	—	—
2.0	146	328*	14.0	1.8
2.5	142	—	—	—
3.0	136	318*	20.3*	3.2
4.0	120	304*	26.0	4.0
4.5	117	—	—	—
5.0	115	300*	31.2	4.9
5.5	110	—	—	—
6.0	104	—	33.5*	6.3
7.0	101	—	41.2*	7.1
8.0	99	—	42.5*	—

NOTE:—*Creatine, 0.4396%; sodium hydroxide, N/1; temp., 50.2° C.*

Discussion of Results

Examination of Tables II and III reveals a definite minimum rate of creatinine formation for 0.1 *N* acid; with decreasing strength of acid a secondary maximum occurs at about 0.01 *N* hydrochloric acid. This is well shown by the figures for a 24-hr. period. The data in the two tables are presented graphically in Figs. 1 and 2.

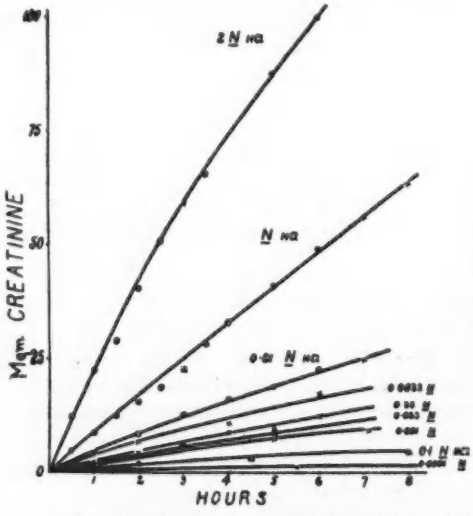


FIG. 1. *Curves illustrating the relative rates of transformation of a 0.4396% solution of creatine into creatinine in different concentrations of hydrochloric acid at 37.5° C. Results in mgm. creatinine per 100 cc. solution. (cf. Table II).*

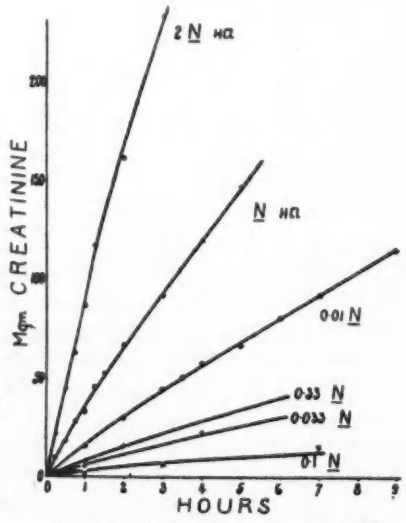


FIG. 2. *As in Fig. 1, but at 50.2° C. (cf. Table III).*

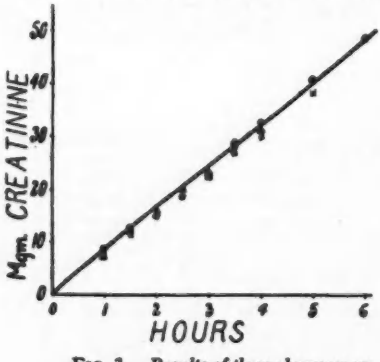


FIG. 3. *Results of three observers on the relative rates of transformation of a 0.4396% solution of creatine into creatinine in N hydrochloric acid at 37.5° C. (Circles represent the mean of three readings by the same observer, crosses and triangles, single readings.)*

(The non-fit of the curve between the 2nd and 4th hours for *N* hydrochloric acid (Fig. 1) appears to be due to experimental error. Fig. 3, recording the readings of three different observers for this strength of acid, does not indicate any definite break in the curve.)

Certain results in the preliminary series, from experiments at 99° C., confirm the minimum observed for 0.1 *N* acid (see Fig. 4); the actual figures from which the curves were drawn require substantial correction for the effect of such a temperature on neutral creatine solutions.

The presence of a minimum rate for 0.1 *N* hydrochloric acid, and a secondary maximum for 0.01 *N*, is illustrated more clearly if the amount of creatinine produced in a definite

time be plotted against the logarithm of the normality of the acid. This has been done for one- and two-hour intervals in Figs. 5 and 6.

The simplest explanation of the apparent anomaly would appear to be that there are two reactions proceeding simultaneously, one of which is much more susceptible to the catalytic influence of the hydrogen ion than is the other. In the presence of hydrochloric acid, creatine hydrochloride or its ionized components are produced. In the presence of excess of acid the transformation is mainly that of the (ionized or unionized) hydrochloride, but when creatine is in marked excess the action must be mainly on creatine itself. The latter is apparently much more susceptible to dehydration than the hydrochloride, since $N/3$ and $N/100$ acid solutions give comparable results. If such a theory be accepted, then decreasing concentration of both hydrochloride and hydrogen ions seems to permit the possibility of that minimum rate which actually occurs with 0.1 N acid.

Accurate determinations of the actual hydrogen ion concentrations in such solutions should determine the soundness of this view. Colorimetric comparisons have been made of the pH in pure acid solutions, and those containing 0.44% of creatine. They are admittedly not very accurate, yet they lend some support. The results are shown in Table VI and also the molar ratios of the solutions employed throughout the experiments; 0.44% creatine is approximately 0.033 molar.

The anomalous results should, if the explanation offered be correct, have a direct bearing on much of the previous work dealing with the velocity constants of this reaction.

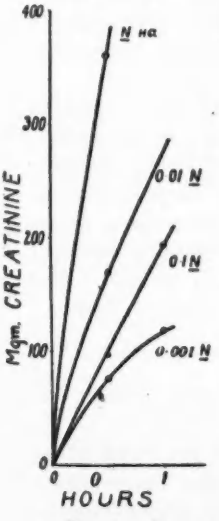


FIG. 4. *Curves illustrating the relative rates of transformation of a 0.4396% solution of creatine into creatinine in different strengths of hydrochloric acid at 99° C. (uncorrected results in mgm. creatinine per 100 cc. solution).*

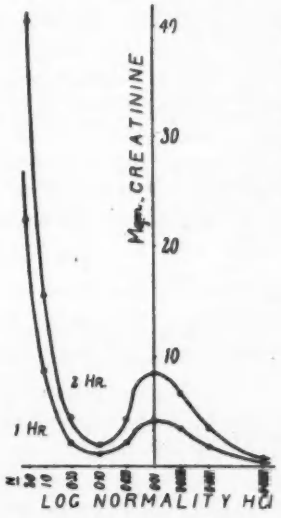


FIG. 5. *Amounts of creatinine (in mgm. per 100 cc. solution) produced in one and in two hours from a 0.4396% creatine solution in different strengths of hydrochloric acid at 37.5° C., plotted against the logarithmic values of the normality of the acid.*

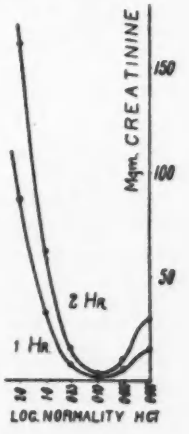


FIG. 6. *As in Fig. 5, but at 50.2° C.*

TABLE VI

MOLAR COMPARISON OF CREATINE AND ACID AND CERTAIN pH VALUES

Normality of HCl	Molar ratio creatine : HCl	Creatinine formed in 1 hr.		Probable main reaction: dehydration of	pH		(H^+)		Molar concentration in creatine-HCl solutions	
		37.5° C.	50.2° C.		Pure HCl	HCl + creatine	Pure HCl	HCl + creatine	Creatine hydrochloride	Free creatine
2.0	1 : 60	22.4	86.8	Creatine-HCl	—	—	—	—	—	—
1.0	1 : 30	8.7	32.8	Creatine-HCl	—	—	—	—	—	—
0.33	1 : 10	2.2	7.4	Creatine-HCl	—	—	—	—	—	—
0.10	1 : 3	1.1	1.5	Creatine-HCl	1.0	1.2	0.1	0.06	0.033	0.000
0.033	1 : 1	2.1	5.1	Creatine	1.5	2.2	0.03	0.006	0.024	0.009
0.010	3.3 : 1	4.1	15.1	Creatine	2.0	3.0	0.01	0.001	0.009	0.024
0.0033	10 : 1	(4.1)	—	Creatine	2.7	3.5	0.002	0.0003	0.0017	0.0313
0.0010	33 : 1	1.7	—	Creatine	3.0	4.2	0.001	0.00006	0.0009	0.0321
0.0001	333 : 1	(0.3)	—	Creatine	4.4	5.7	0.00004	0.000003	0.00004	0.03296

The validity of such work would appear to be rendered questionable for concentrations of acid above 0.01 *N* (cf. Hunter (13)).*

Figs. 1, 2, and 7 indicate that with increasing concentration of acid (above 0·1 *N*) or of creatine there is a relatively greater increase in rate of reaction, results in accordance with usual findings for such reactions.

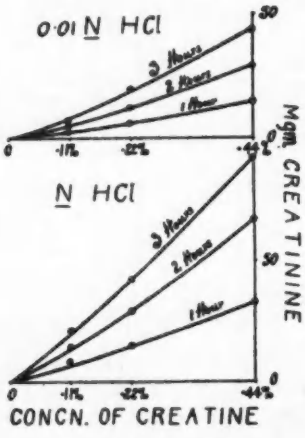


FIG. 7. *Curves illustrating the relatively greater increase in speed of transformation, with increasing concentration of creatine. Results in mgm. creatinine per 100 cc. solution.*

For the determination of the Arrhenius constant accurate series of determinations are available for 2 *N*, *N*, and 0.01 *N* hydrochloric acid. Table VII shows that the results are in reasonable agreement with those for a monomolecular reaction. The Arrhenius constant is given by the equation

$$\mu = 4.6 \times \frac{(\log k_2 - \log k_1)}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where k_1 and k_2 are the velocity constants at the absolute temperatures T_1 and T_2.

The summarized figures for the constant for 0.4396% of creatine and the temperatures 37.5° and 50.2° C. are as follows:—for 2 *N* hydrochloric acid, μ equals 24.0×10^3; for *N* acid, 23.2×10^3; and for 0.01 *N* acid, 20.7×10^3.†

Edgar and Wakefield's figures (7), with varying concentrations of creatine, are as follows:—for 25° and 57° C. and 0.38 *N* hydro-

*According to Hahn and Fasold (9), there is a somewhat similar complexity in alkaline solutions of creatine. The alkaline salts which are formed hydrolyse much more slowly than does the free creatine. Figs. 8 and 9 also suggest such complexity.

†See Cameron (4, p. 59) for a discussion of the significance of the form of expressing μ.

TABLE VII

REACTION VELOCITIES AND ARRHENIUS CONSTANTS FOR THE CREATINE-CREATININE REACTION

Time, hr.	2 *N* Hydrochloric acid				*N* Hydrochloric acid				0.01 *N* Hydrochloric acid			
	37.5° C.		50.2° C.		37.5° C.		50.2° C.		37.5° C.		50.2° C.	
	Residual creatine	*k*	Residual creatine	*k*	Residual creatine	*k*	Residual creatine	*k*	Residual creatine	*k*	Residual creatine	*k*
0.25	—	—	414.4	.1026	—	—	428.5	.0444	—	—	—	—
0.50	425.2	.0289	388.4	.1076	434.3	.0105	419.2	.0413	—	—	—	—
0.75	—	—	366.2	.1058	—	—	407.6	.0437	—	—	—	—
1.00	414.6	.0265	338.9	.1130	429.5	.0101	401.6	.0393	434.8	.00477	422.1	.017(
1.25	—	—	304.1	.1280	—	—	386.4	.0448	—	—	—	—
1.5	406.3	.0228	—	—	425.1	.0097	378.6	.0432	—	—	—	—
2.0	393.0	.0243	252.4	.1205	421.6	.0091	362.1	.0421	429.7	.00494	405.0	.0178
2.5	380.7	.0250	—	—	417.9	.0088	—	—	—	—	—	—
3.0	371.2*	.0245	168.5	.1388†	413.4	.0089	332.6	.0404	425.0	.00489	388.2	.0180
3.5	363.9	.0234	—	—	407.0	.0096	—	—	—	—	381.0	.0178
4.0	—	—	—	—	401.4	.0099	300.9	.0412	420.7	.00477	373.0	.0178
5.0	338.3	.0227	—	—	392.0	.0099	268.8	.0427	417.7	.00444	362.0	.0169
6.0	323.7	.0221	—	—	382.9	.0100	—	—	413.4	.00447	345.6	.0174
7.0	—	—	—	—	375.0*	.0099	—	—	411.2*	.00414†	332.1	.0174
8.0	—	—	—	—	366.4*	.0099	—	—	—	—	—	—
9.0	—	—	—	—	—	—	—	—	—	—	305.3	.0176
	Mean	.0245	Mean	.1129	Mean	.0097	Mean	.0423	Mean	.00471	Mean	.0176
	$\mu = 24.0 \times 10^3$				$\mu = 23.2 \times 10^3$				$\mu = 20.7 \times 10^3$			

**Single determination.* *†Excluded in determination of mean.*

chloric acid, μ equals 19.05×10^3; for 57° and 78° C. and 0.38 *N* acid, 20.4×10^3; for 78° and 100° C. and 0.38 *N* acid, 20.5×10^3; and for 78° and 100° C. and 0.19 *N* acid, 19.8×10^3.

The data presented are scarcely sufficient to stress the differences obtained with weak and with strong acid, although such differences may well be real, indicating different constants for the dehydration of creatine hydrochloride and of creatine itself.

Since the conversion of creatine into creatinine is catalyzed by both hydrogen and hydroxyl ions theoretically the reaction affords an opportunity of contrasting the Arrhenius constants for these two agents under similar conditions. Preliminary determinations showed, however, that a prolonged study of the complex reaction in alkaline solution will be essential before such a contrast can be made. These preliminary studies are recorded in Figs. 8 and 9 (in which the continuous lines represent subsequent accurate determinations). Some results bearing upon the general decomposition of creatine in alkaline solution will be recorded.

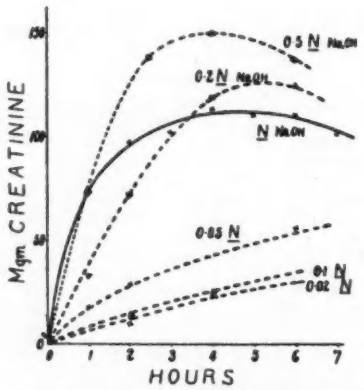


FIG. 8. *Curves illustrating preliminary studies of the rate of transformation of a 0.4396% creatine solution in different strengths of sodium hydroxide at 37.5° C. Results in mgm. creatinine per 100 cc. solution.*

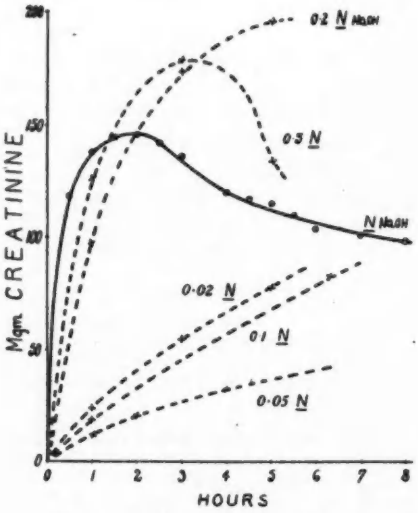
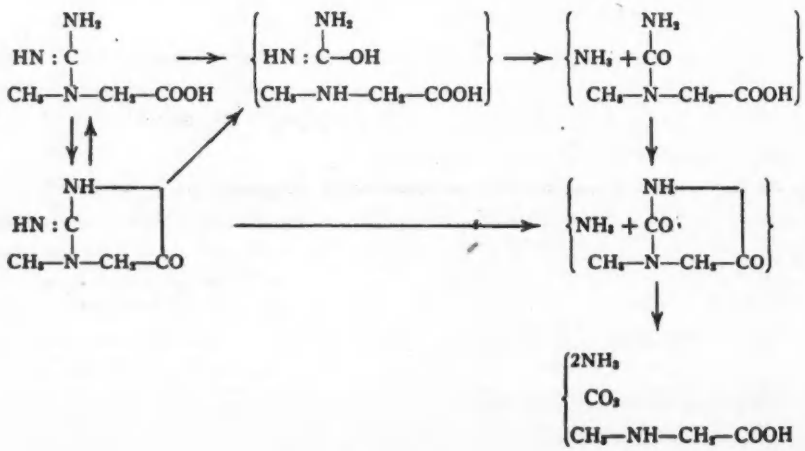


FIG. 9. *As in Fig. 8, but at 50.2° C.*

According to Gaebler (8) the initial decomposition of creatinine (or of creatine) by alkali is into urea and sarcosine, which then reunite to form ammonia and methyl hydantoic acid, methyl hydantoin being subsequently formed. (Collip's isolation of methyl hydantoin from testicular material may, from this point of view, indicate that it is formed in the organism from creatine, in which testicular material is relatively rich.) In accordance with Gaebler's views, the decomposition of creatine may be represented by the scheme:



From the results in Table V (and their graphical representation in Fig. 10) it is evident that the formation of ammonia is much slower than that of urea, in support of Gaebler's views. These results also indicate that the apparently rapid slowing down of creatinine formation, shown in the curves of Figs. 8 and 9, is but little due to the formation of urea and sarcosine (and ammonia and methyl hydantoic acid), but is mainly to be attributed to the relatively more rapid conversion of creatinine into creatine. Bearing this in mind, the results exemplify a point that is seldom stressed, that in solutions of acid and of alkali of corresponding strength the alkali catalyzes the change from creatine to creatinine much more rapidly than does the acid.

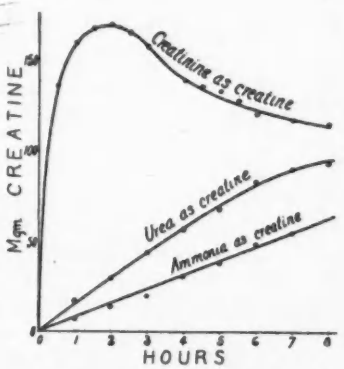


FIG. 10. *Curves illustrating the relative rates of formation of creatinine, urea, and ammonia in a 0.4396% solution of creatine, and in N sodium hydroxide solution, at 50.2° C. All results are expressed in terms of creatine and per 100 cc. solution.*

When the results are presented (Table VIII) in such form that all figures for creatinine, for urea (representing the urea and sarcosine stage), and for ammonia (representing the final stages), are expressed in terms of creatine, the total agrees within the limit of error with the original amount of creatine, so that any changes not included in the above scheme of reactions can occur only to a negligibly small extent. Fig. 11 therefore summarizes in graphical form the reaction in alkaline solution for the conditions stated.

TABLE VIII

SUMMATION OF RESULTS OF ACTION OF *N*/1 NaOH ON CREATINE AT 50.2° C.

Time, hr.	0.5	1	2	3	4	5	6	7
Residual creatine	274	243	211	212	214	215	(198)	(178)
Creatinine as creatine	136	159	169	157	139	133	120	117
Urea as creatine	9*	17	30	44	57	68	73	90
Ammonia as creatine	4*	7	14	25	31	38	49	55
Total as creatine	423	426	424	438	441	454	—	—
Total creatine used	440	440	440	440	440	440	440	440
Error	−17	−14	−16	− 2	+ 1	+14	—	—
Error, %	−3.9	−3.2	−3.6	−0.5	+0.2	+3.2	—	—

**Calculated from curves.*

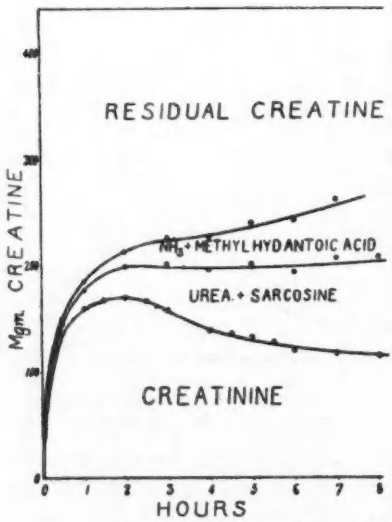


FIG. 11. *Scheme summarising the transformation of creatine (0.4396% solution) in N sodium hydroxide solution at 50.2° C. Results calculated in terms of creatine and per 100 cc. solution.*

The results of Cannan and Shore (5) and those of Hahn and Meyer (10, 11) appeared to give a satisfactory explanation for the conversion of creatine into creatinine in muscle, provided muscle creatine were free. The discovery of phosphocreatine has rendered such calculations invalid. The present results are insufficient to permit definite conclusions but give a slight indication that even at the hydrogen ion concentration in fatigued muscle the rate of formation of creatinine (as indicated by its rate of excretion), if due simply to catalysis, by hydrogen ions, of such free creatine as is transiently present, is insufficient to account for the amount actually excreted.

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THE INFLUENCE OF ELECTROLYTES ON THE FORMATION AND DECOMPOSITION OF URATE GELS[1]

By E. Gordon Young[2], F. F. Musgrave[3] and H. C. Graham[3]

Abstract

A study has been made of the influence of various electrolytes in promoting gelation of aqueous supersaturated solutions of the urates of methylamine, lithium, piperazine and tetramethylammonium. The cation has been found to be the active agent. The minimal concentration of the electrolyte which is effective has been determined over the gelation range of the urate. The order of efficacy of the cations may be stated in general to be $K > NH_4 > Rb > Cs > Li > Na$. A maximal concentration of electrolyte has been found above which immediate precipitation rather than gelation occurs. The time required for gelation varies inversely with the amount of electrolyte present in any single concentration of urate. The rigidity varies directly up to the point of precipitation. The time of crystallization of the gel varies *directly* with the concentration of the electrolytes, sodium chloride, rubidium chloride and lithium chloride but *inversely* with potassium chloride and ammonium chloride. Certain organic diamines have been found to possess the power of causing gelation over a limited range of urate. Ethyl alcohol, between 20 and 60%, causes gelation of lithium urate and methylamine urate.

The thixotropic effect has been observed in gels of piperazine urate containing potassium chloride and lithium urate with alcohol.

The action of electrolytes on urate gels is interpreted as partially electrokinetic and partially lyotropic in nature.

It has been observed by Schade and Boden (9) that supersaturated solutions of sodium urate may be aided in forming gels by the addition of concentrated solutions of certain common salts. They have used sodium and potassium chlorides, ammonium, magnesium and sodium sulphates and trisodium phosphate. While investigating the influence of physical and chemical conditions on the preparation of various urates as gels the importance of the presence of electrolytes was confirmed in this laboratory (12). Two classes of urates were found; one spontaneously forming gels on cooling, a temperature-gelation effect; the other forming gels only in the presence of electrolytes, an electrolyte-gelation effect. Because of the general distribution of electrolytes in biological material, the possible importance of this effect in nature is apparent. Particularly is this so in the light of the occurrence and unusual behavior of abnormally high concentrations of uric acid in blood and urine (3, 8, 11). A more detailed study of this effect was therefore undertaken using two examples of urates which form gels spontaneously, methylamine and lithium urates, and two which do not, tetramethylammonium and piperazine urates.

The general plan of the investigation has been to study the influence of various concentrations of electrolyte on supersaturated solutions of urate below the concentration at which gels form spontaneously. The minimal

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[2] *Professor of Biochemistry, Dalhousie University.*
[3] *Banting Research Foundation Grantee.*

value of the electrolyte which would induce a thin gel at a particular concentration of urate has been noted for comparative purposes. In other series the maximal value allowing gels to form has been observed also. Finally the influence of certain non-electrolytes which behave as dehydrating agents has been determined in an effort to explain the mode of action of the electrolyte in gel formation.

Experimental

Methylamine Urate

Methylamine was selected as the first base with which to make urate preparations because of the ease with which supersaturated solutions were formed and the short duration of the gel state before crystallization took place. The limits of concentration forming gels have been determined by the writers to be 1.5–10%, and the time required for crystallization from a few minutes up to 24 hr. at 20° C. depending upon concentration.

In the earlier experiments a definite concentration below that at which gels would form in pure solution was adopted and the effect of a large number of electrolytes observed. In the later experiments the range of concentrations of urate which could be induced to gel by electrolytes was determined using a small selected group of salts.

The procedure was to suspend 0.3 gm. of pure crystalline uric acid in 50 cc. of distilled water previously heated to about 90° C., to add a few drops of phenol red (0.02%), and an aqueous solution of methylamine (33%) drop by drop until the uric acid had dissolved completely and the pH was approximately 7. This clear solution was rapidly divided into fractions of 10 cc. each, varying quantities of a normal solution of electrolyte were added and the tubes cooled in an ice bath. The criterion of gel formation was judged by the rigidity of the solution on placing the test tube horizontally. This is very approximately equivalent to accepting a particular viscosity as end point to be attained in all preparations. More exact technique would be difficult because of the nature of urate gels and their non-elasticity.

The conditions adopted were the result of previous experience. It is essential that the pH be carefully adjusted because, at the lower concentrations of methylamine urate, gelation will readily take place only in the narrow range, 6.8–7.3. In such cases where the electrolyte added was either acidic or basic, allowance had to be made for this in the initial pH so that the final pH of the solution would be approximately 7. During the formation of the gel the pH shift to the alkaline side is observable as previously recorded (12).

At a concentration of 0.6% uric acid the following salts of potassium were found to induce gelation,—iodide, chloride, nitrate, chromate, sulphate, primary and secondary phosphates; of ammonium, the chloride, nitrate, chromate, sulphate, primary and secondary phosphates. The writers have been unsuccessful in causing gelation in the presence of salts of potassium or ammonium with weak acid radicals such as arsenate, arsenite, citrate or borate. Tertiary phosphates are difficult to use because of their basicity and

influence on the final pH. Salts of the heavier metals such as barium, calcium, magnesium and aluminium, caused immediate precipitation presumably of the respective urate.

In Table I are recorded as millimoles per litre the effective salts of potassium and ammonium in the minimal concentration at which they have caused gelation of methylamine urate in 0.6% solution. The time required for setting is also listed although this varies considerably and depends upon the amount of electrolyte present. The "potassium" gels were more stable than the "ammonium" gels. It is apparent from Table I that there is no difference in the efficacy of the anions Cl, I, NO_3, SO_4, CrO_4, PO_4. From the concentrations of electrolyte required it may be concluded that the active ion is the cation and presumably the urate micelle is negatively charged. This is in agreement with the conclusion of Keeser and Zocher (4) from different experimental data.

TABLE I

GELATION OF METHYLAMINE URATE

Electrolyte	Minimal conc., millimoles/litre	Time of gelation, min.	Electrolyte	Minimal conc., millimoles/litre	Time of gelation, min.
Ammonium chloride	25	20	Potassium chloride	25	30
Ammonium nitrate	25	15	Potassium nitrate	25	180
Ammonium sulphate	12	25	Potassium sulphate	12	120
Ammonium chromate	12	90	Potassium chromate	12	300
Ammonium phosphate (primary)	8	120	Potassium phosphate (primary)	8	120
Ammonium phosphate (secondary)	7	240	Potassium phosphate (secondary)	8	120
Potassium iodide	25	60			

Selecting the chlorides as suitable salts a study was next made of the concentration of electrolyte required to cause gelation in decreasing concentrations of urate. The technical mode of procedure was as previously stated. Table II shows the values in millimoles for potassium, ammonium, lithium, sodium, rubidium, and caesium chlorides.

TABLE II

EFFECT OF ELECTROLYTES ON GELATION OF METHYLAMINE URATE

Uric acid, %	KCl	NH_4Cl	RbCl	NaCl	CsCl	LiCl
1.2	3	5	10	15	60	100
1.0	4	10	20	40	150	300
0.8	9	20	40	100	300	500
0.6	25	50	100	200	?	700
0.4	100	150	200	300	?	1250
0.3	150	200	—	—	?	—
0.2	—	—	—	—	?	—

Marked differences are apparent in the concentration of salt required to produce the same effect. It is evident in all cases that with decreasing concentrations of urate increasing amounts of electrolyte are required to attain the gel state. The concentration at which this power is lost also depends upon the particular electrolyte. The experiments with caesium chloride are incomplete because of the high cost of this salt, but they are sufficient to indicate the position of caesium in the group. The results are expressed graphically in Fig. 1 where the positions of the various salts are clearly shown.

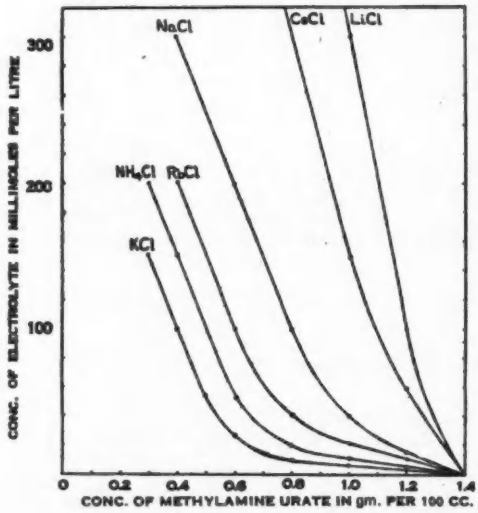

FIG. 1. *The comparison of the minimal concentrations of various electrolytes required to induce gelation of solutions of methylamine urate.*

Potassium chloride is taken to be the most effective salt and its action extends down to a concentration of uric acid of 0.3%. The action of ammonium chloride is very similar. "Ammonium" gels tend to form more rapidly and crystallize more rapidly than "potassium" gels. The amount of sodium chloride required for a particular concentration of urate is more difficult to determine than that of any other salt because of rapid crystallization of the gel and the tendency of very weak gels to form. Lithium chloride is required in much the highest molar concentration to induce gelation. The order of efficacy of the cations of this group may therefore be placed as follows: $K > NH_4 > Rb > Na > Cs > Li$.

Lithium Urate

The behavior of electrolytes in a supersaturated solution of lithium urate was next studied to determine whether the observed effects were applicable to other urate solutions. Lithium urate provided a gel which was much more stable than those of methylamine, forming spontaneoulsy at 0° C. at a concentration of 2–5% and at a pH of 6–12. The procedure was slightly modified from the preceding one.

A definite weight of uric acid was suspended in water at 80–90° C., dissolved with 1 *N* lithium hydroxide, the pH adjusted to 7, the solution made up to volume and filtered if necessary. Five cubic centimetres of this solution was added to 5 cc. of electrolyte previously warmed to 50° C., the test tube was shaken and placed immediately in an ice bath. The original solutions were made double the concentration required in the experiment.

In this series the chlorides only of the alkali metals were used. The observations were extended to include the behavior of gels in the presence

of varying amounts of electrolyte. Below the minimum required for gelation the solution remained perfectly clear with or without an increase in viscosity until the time that crystallization began. Above the maximum an amorphous or crystalline deposit formed before gelation could take place.

In Table III are recorded in millimoles per litre the concentrations observed at definite levels of lithium urate.

TABLE III

EFFECT OF ELECTROLYTES ON GELATION OF LITHIUM URATE

Uric acid, %	KCl		NH_4Cl		NaCl		LiCl	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
2.0	0	1000	0	100	0	500	0	2400
1.8	0.3	1000	0.3	100	10		10	
1.0	10	1000	10	100	150	1000	150	4250
0.5	80	1500	80	200	1500	2000	400	4250
0.3	200		200		—		1250	
0.1	1500	2700	—		—		—	

NOTE:—*Concentrations of electrolytes expressed in millimoles per litre.*

Considering the minimal values it is evident that, as in the case of methylamine urate, potassium chloride is the most effective agent promoting gelation in a concentration of uric acid as low as 0.1%. This is, to the authors' knowledge, comparable with the lowest concentration of any solute in the gel state. The results with ammonium chloride are identical except that its influence does not extend to quite as low a concentration of urate. The inverse relation of concentration of electrolyte to concentration of urate likewise holds as with methylamine urate. The order of efficacy of the cations in this series would therefore be as follows: $K > NH_4 > Li > Na$. The results are plotted in Fig. 2. It is observable that in comparison with Fig. 1 the slopes of the curves are somewhat sharper at the lower concentrations of urate.

Considering the maximal values it is to be noted that, with the exception of ammonium chloride, the concentrations required to promote immediate precipitation are relatively high. Expressed in terms of percentage for the action on a

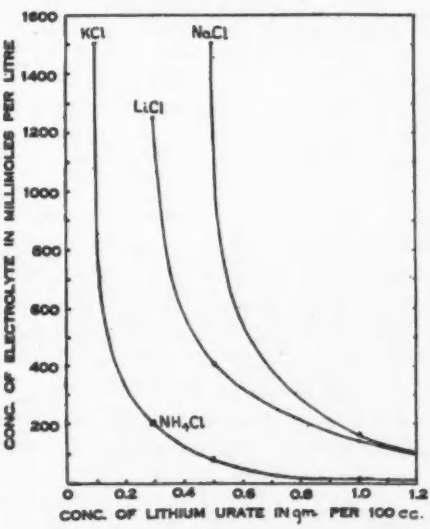

FIG. 2. *The comparison of the minimal concentrations of various electrolytes required to induce gelation of solutions of lithium urate.*

1% solution of lithium urate the figures are as follows (Table IV), compared with saturation values at 0° C.

TABLE IV

Concentration	NH_4Cl	KCl	NaCl	LiCl
At gelation maximum	0.54	8.2	5.8	18.0
At saturation (0° C.)	29.4	27.6	35.7	67

Tetramethylammonium Urate

Tetramethylammonium hydrate provides an example of a substance which forms a urate that is readily soluble. It is comparable with lithium hydroxide in its action but differs in that the viscosity of such solutions is not materially increased on cooling and no gels are formed. This urate is therefore a good example with which to test the action of electrolytes. The technique has been essentially the same as described under lithium urate. A 10% solution of the base has been used. The concentration of urate has been limited to 2% or less and the variety of electrolytes to the chlorides of the commoner alkali metals. No higher concentration than 2% of the urate is recorded because it was found that the concentration of electrolyte required was very similar. The results are recorded in Table V as minimal values in millimoles per litre of the electrolyte required to induce gelation at the particular concentration of urate.

TABLE V

EFFECT OF ELECTROLYTES ON GELATION OF TETRAMETHYLAMMONIUM URATE

Uric acid, %	KCl, 50° C.	NH_4Cl, 0° C.	LiCl, 0° C.	NaCl, 50° C.
2.0	20	5	200	500
1.0	30	10	300	1250
0.5	100	20	750	2500
0.3	300	30	1250	—
0.1	1200	—	—	—

The same general result was observed as in the two previous examples. Potassium chloride was effective in the greatest dilution of urate and the results are comparable with those for lithium urate. Ammonium chloride acted in the lowest molar concentration. Its action is one of precipitation rather than gelation below 0.3% urate. It was found in the case of this salt that the temperature of the solution of electrolyte to which the supersaturated urate was added was an important factor. Thus if the initial temperature was 50° C., as previously, a voluminous amorphous precipitate resulted on adding urate, but if 0° C. a gel formed. This was also found true for lithium chloride. It will be noted that the two salts act over the same range but at very different molar concentrations. Sodium chloride is the least effective salt as with lithium urate. The order of efficacy of the cations for this series would therefore be $K > NH_4 > Li > Na$.

Piperazine Urate

Another example of a urate in the class of electrolyte-gelation is that of piperazine. This moderately weak base has the power of dissolving uric acid readily and forming supersaturated solutions up to about 7% at 90° C. The solubility of piperazine urate at 20° C. is about 0.5%. The technique used was the same as with lithium urate. Piperazine hydrate was added as the solid in the formation of the initial solution. The chlorides of rubidium and caesium were included in this series. In Table VI are summarized the results. The initial temperature of the electrolyte was 50° C. in all cases except ammonium chloride. In order readily to obtain gels with the latter it was found necessary to cool both electrolyte and urate solutions to about 0° C. before mixing.

TABLE VI

EFFECT OF ELECTROLYTES ON GELATION OF PIPERAZINE URATE

Uric acid, %	KCl	RbCl	NH_4Cl	CsCl	LiCl	NaCl
4.0	3	0.5	1	10	100	100
2.0	5	10	5	100	300	300
1.0	50	40	100	300	500	1000
0.5	200	100	200	500	1500	2000
0.3	400	300	300	?	—	—
0.1	1200	1200	1200	?	—	—

The cations in these experiments may be divided into three groups. The efficacies of potassium, rubidium and ammonia are about the same. Caesium holds an intermediate position. The full range of caesium chloride was not determined because of a scarcity of material, but the results obtained are sufficient to fix its position without doubt. Lithium and sodium are very similar with the former slightly more effective. The order would thus be $K = Rb = NH_4 > Cs > Li > Na$.

Rate of Formation and Stability of Gels

Throughout the experiments recorded above the intervals of time required for the formation of the gel state and the duration of the latter have been observed. The gel is broken by the crystallization of the urate which becomes microscopically or macroscopically visible. This may take place in relatively few minutes or it may require many days depending on the particular urate and electrolyte used. Without exception it has been observed that the time required for gelation varies inversely with the amount of electrolyte present in any single concentration of urate. This has been found true for all electrolytes in all preparations of the different urates. This statement is illustrated numerically in Table VII. It was observed also that the rigidity of the gel is directly proportional to the concentration of electrolyte present in any single concentration of urate up to the point of precipitation rather than gelation. These facts are of some help in interpreting the meaning of the maximum figures in Table III.

Records have also been kept of the time intervals of stability of the gels before crystallization destroyed the rigidity measured by the authors' rough method. Typical results are given in Table VII.

TABLE VII

INFLUENCE OF ELECTROLYTES ON RATES OF GELATION AND CRYSTALLIZATION

Lithium urate, 2%			Lithium urate, 0.5%		
Conc. NaCl, millimoles	Gelation, sec.	Crystallization, hr.	Conc. KCl, millimoles	Gelation, sec.	Crystallization, min.
100	90	1	100	420	100
200	60	3	300	150	85
300	30	8	500	90	70
400	5	10	900	60	55
500	2	12	1500	15	45
Piperazine urate, 2%			**Piperazine urate, 3%**		
Conc. LiCl, millimoles	Gelation, min.	Crystallization, hr.	Conc. RbCl, millimoles	Gelation, min.	Crystallization, min.
300	20	> 24	2	17	45
500	9	> 24	6	8	50
750	7	> 24	10	5	60
1250	5	> 24	20	3	75
Tetramethylammonium urate, 0.3%					
Conc. NH_4Cl, millimoles	Gelation, sec.	Crystallization, min.	Conc. NH_4Cl, millimoles	Gelation, sec.	Crystallization, min.
100	360	20	500	10	2
300	20	8	900	2	1

It is evident that the time of crystallization varies *directly* with the concentration of the electrolytes, sodium chloride, rubidium chloride and lithium chloride, but *inversely* with potassium chloride and ammonium chloride. This has been found true for the four different preparations of urate used. Furthermore, the greater the concentration of urate at any one concentration of electrolyte the more rigid is the gel. The gels formed with lithium chloride are particularly stable, breaking up very slowly by the formation of numerous macroscopically visible nuclei in the course of days or weeks. The original gel can be melted and reformed by cooling to 0° C. if the concentration of urate is low. If the latter is high, melting is a slow procedure even at 100° C. Crystallization of these gels likewise is very slow to develop, frequently requiring many weeks. For this reason it is not practicable to include in Table VII times of crystallization for this electrolyte.

Effect of Diamines

The action of electrolytes in causing gelation of solutions of urates is probably one of coagulation of colloidal particles. If conditions are favorable a gel results, if not, a precipitate. The explanation of this coagulation is possible in two ways. The phenomenon may be electrokinetic in nature and as such it should conform to the facts of coagulation of typical sols and the Schulze-Linder-Picton law pertaining to valence. The curves illustrated in Figs. 1 and 2 are very similar to those obtained by Burton and Bishop (1) for the coagulation of sols of gum mastic and arsenious sulphide by univalent ions.

It has been possible in the experiments recorded above to study the efficacy in promoting gelation of cations of univalent metallic salts only. Polyvalent metallic ions have in all cases tried caused immediate precipitation of the corresponding very insoluble urate. In order to determine the effect of divalent ions it was therefore necessary to use the chlorides of soluble diamines. At a concentration of 1.8% of lithium urate, or just below the normal gelation minimum, three were effective in causing gelation as indicated below. Quinine hydrochloride was without effect. The results with a univalent amine, aniline chloride, and the chlorides of the alkali metals are also listed in Table VIII for comparative purposes. The numerical values express concentration in millimoles per litre.

TABLE VIII

Phenylenediamine	0.5	NaCl	10
Benzidine	1	LiCl	10
Ethylenediamine	2	KCl	0.3
Aniline	10	NH_4Cl	0.3

This influence of the diamines is limited to a narrow range of concentration of the lithium urate.

It is thus apparent that although the diamines are effective they are no more so than univalent ions. The results do not suggest a simple application of the Schulze-Linder-Picton law to the formation of gels. The influence of concentration of the colloid must however be taken into account as pointed out by Burton and Bishop (1). It should further be emphasized that the inverse relation of concentration of urate to concentration of electrolyte is at variance with the usual direct relation as exhibited by similar sols such as stannic hydroxide, manganese dioxide, aluminium hydroxide, vanadium pentoxide and thorium hydroxide (2). There are however exceptions to the latter rule.

Effect of Non-electrolytes

The phenomenon is also explicable on the basis of a lyotropic effect and it should be expected both to find non-electrolytes active in promoting gelation and to observe the lyotropic series in the efficacy of the cations studied. A summary of all the results pertaining to this point is given below:—

Methylamine series, $K > NH_4 > Rb > Na > Cs > Li$; piperazine series, $K = NH_4 = Rb > Cs > Li > Na$; lithium series, $K > NH_4 > Li > Na$; tetramethylammonium series, $K > NH_4 > Li > Na$; lyotropic series, $Li > NH_4 > Na > K > Rb > Cs$.

With the exception of the position of sodium in the methylamine urate series the efficacy of the ions is essentially the same in all types of urate employed,—qualitatively but not quantitatively. The order bears no resemblance to that of the lyotropic series or hydration capacity of the ions.

A further method of testing the nature of the phenomenon in supplement to those described is the effect of non-electrolytes upon gelation. Schade and Boden (9) have observed that the presence of alcohol in 50% concentration will aid the gelation of supersaturated sodium urate. Keeser and Zocher (4) have found that the addition of non-electrolytes such as urea, glucose, sucrose, glycerol and ethyl alcohol decreased the rigidity of the gels with increasing concentration and retarded the crystallization generally. Urea however was found to accelerate crystallization. The type of influence on the rigidity of the gels is the opposite of the writers' observations for electrolytes.

As a means of testing whether non-electrolytes possessed the power of causing gelation, alcohol was selected. Its effect was tested in the same way as previously with solutions of electrolytes. Double the concentration of the urate desired was added to various concentrations of ethyl alcohol at 50° C. and the mixture cooled immediately in an ice bath. The concentration of urate employed has been on the limits at which gels would form spontaneously. The effect of alcohol on the gelation of 1.7% lithium urate is shown in Table IX.

TABLE IX

EFFECT OF ALCOHOL ON GELATION OF LITHIUM URATE

Concentration of alcohol, %	Result
10	No change in 24 hr.
20	Gelatinous ppt. becoming weak gel
30	Gelatinous ppt. becoming weak gel
40	Firm gel in 2 hr.
50	Firm gel in 1.5 hr.
60	Firm gel in 1 hr.
70	Crystalline ppt. immediately

It will be seen that alcohol in this experiment acted in a similar way to solutions of electrolytes. In low concentration the effect was not apparent; in intermediate concentration gels were formed. The rigidity and speed of formation increased with increasing amounts of the active agent; in high concentration immediate precipitation of the urate occurred. The phenomenon of gelation in alcohol is interesting. An opalescence first appears which soon gives place to a turbidity or micro-coacervation which in turn becomes a gelatinous precipitate or macro-coacervation. The latter passes into a firm clear gel which in turn breaks down slowly depositing crystals. The less rigid gels in the lower concentrations of alcohol exhibit the phenomenon of thixotropy. The writers have also observed this phenomenon in gels of piperazine urate formed with potassium chloride. These are the only instances amongst urate gels where they have observed this effect.

As applied to lower concentrations of lithium urate the method employed yielded gelatinous, then amorphous, precipitates rather than gels. With 2% methylamine urate the results were similar to those in Table IX. With the urates of piperazine and tetramethylammonium hydrate only precipitates

were formed. In general it may be stated that those urates which form gels spontaneously will do so in the presence of alcohol but that alcohol *per se* has little or no power to promote gelation. Other non-electrolytes which were tried have been without any influence on the phenomenon of gelation. Thus urea, glucose, glycol, and glycerol were without any effect as were also the colloids, gelatin, ovalbumin and gum arabic. In high concentration some tend to cause a precipitation of the urate. Their influence on the stability of gels was not studied.

Sol-gel-sol Transformation

In many experiments the writers observed the appearance of a slight opalescence in their preparations on cooling in the ice bath prior to the gelation of the solution. This phenomenon is usual in concentrated solutions of urates to which no electrolyte has been added, but not in such dilute solutions as have been employed in these experiments with electrolytes. Not only does a slight opalescence appear but this is of a graded character with increasing concentration of electrolyte. Such sols pass into gels as described above. In those experiments in which ammonium chloride has been used as electrolyte the gels break up in the course of a few minutes with the formation of brilliantly opalescent sols. A slight crystalline precipitate is deposited in the course of days, weeks or months, and the opalescence of these solutions slowly disappears by sedimentation in the course of many months. Examination of these opalescent solutions reveals them microscopically empty but ultramicroscopically brilliant with ultramicrons. Such a result is strongly suggestive of the observations which have been made on agar sols under the influence of alcohol whereby they pass from an emulsoid to a suspensoid type of colloid (5, 6).

Discussion

The main purpose of this investigation was achieved in the demonstration that dilute solutions of urates would gel in the presence of moderate concentrations of such common electrolytes as the chlorides of sodium and potassium. The lowest concentration of uric acid which would gel according to the writers' standard was 0.1%, but it must be emphasized that solutions of considerable viscosity exist below this concentration. It has been shown elsewhere that the blood and urine of the bird, if not of the mammal, may attain this level of uric acid concentration. The urine of the bird is normally considerably above it. The concentration of electrolyte required to induce a colloidal condition therefore becomes of importance. It may legitimately be questioned at this time whether the conditions studied have any direct application to mammalian tissues. They must have to the physical conditions of urate which exist in birds and reptiles.

A further point of interest lies in the similarity of behavior of urate solutions and the hydrous oxides such as ferrous oxide, vanadium pentoxide, alumina, silica, molybdenum pentoxide, ceria, chromic oxide in their response

to electrolytes. It is natural to ask (*a*) the nature of the mechanism whereby urate gels are formed, and (*b*) whether the present experiments have anything new to offer to the general conceptions of gel structure.

In attempting to answer the first question it is necessary to balance the evidence submitted in the experimental section. The action of electrolytes may be interpreted in two ways. It is possible to consider the state of gelation as intermediate between a supersaturated solution containing particles in both molecular and colloidal dispersions and an amorphous or crystalline precipitate—and therefore a stage in coagulation. At a given concentration of urate, it is possible by gradually increasing the amount of electrolyte to pass from a clear stable supersaturated solution which slowly deposits crystals through increasingly viscous sols and increasingly rigid gels to instantaneous amorphous precipitation. This evidence in itself the writers consider as support for the explanation as advanced by Usher (10), that a gel is a transitional stage in the formation of the precipitate. It may be considered as electrokinetic in nature as was pointed out above. The general relation of concentration of urate to concentration of electrolyte required to form a gel conforms to the behavior of electrolytes in the precipitation of such sols as those of copper, arsenious sulphide and gum mastic (1). Thus for univalent ions the concentration of ion necessary to produce a gel increases with decreasing concentration of urate very rapidly at low concentrations of the colloid. As against such a simple explanation of the phenomenon there is the anomalous behavior of the divalent ions of the diamines. These are quite effective but no more so than certain univalent ones and act over a more limited range of urate concentration.

The meaning of the maximal values recorded in Table III becomes clearer if the above explanation of gelation is accepted. A point is reached in the concentration of electrolyte beyond which the coagulation of colloidal particles is so rapid and uneven that amorphous masses result rather than regular and slower precipitation as a network with enclosed solvent or solution. That such a network exists in the gel structure is well illustrated from work with preparations of urates, where all stages of structure may be observed from viscous solutions which are almost gels and fragment on disturbance to coarsely amorphous precipitates formed throughout the liquid, from which solution may be caused to be liberated by mechanical agitation.

The behavior of the gels from the standpoint of a hydrophylic colloid may also be considered. In such a concept it would be the lyotropic power of the cation or electrolyte which would bring about the coagulation of partially dehydrated particles. As has been pointed out above the order of cations in these studies does not coincide with the lyotropic series. On this fact alone it would seem difficult to defend this explanation of urate gelation without qualification. There is further evidence on this point from the present experiments with alcohol. As shown in Table IX alcohol can act in a similar manner to the electrolytes but to a much more limited degree. It must also be remembered that we are dealing with a colloidal electrolyte itself. Furthermore alcohol has been found to act in only one of the four

urate preparations. Its behavior is not comparable for example with its action on agar sols in which the viscosity is markedly decreased with increasing alcohol strength and the preparation takes on the behavior of a suspensoid (5, 6). The action is more easily understood as comparable to the behavior of cerium oxide with alcohol (7) in which the alcohol forms alcogels and does not act primarily as a dehydrating agent.

In attempting to arrive at a picture of the gel mechanism for urates the writers would call attention to the statement of Kruyt and van der Made (7) that "only hydrated sol particles are able to cause gelation phenomena . . . . that it is due to the clotting together of hydrated particles with water imprisoned between them, and that this clotting together has the character of a coagulation. It is thus a coagulation of hydrated particles. For this coagulation either the addition of a discharging electrolyte or the removal of the peptising electrolyte is required". From the above discussion it would appear that we are dealing with a case intermediate between suspensoid and emulsoid in which the character of the suspensoid is predominant, and suggestive of the behavior of the gels of alumina, molybdenum pentoxide and vanadium pentoxide; but that there are two factors of stability—hydration and ionic adsorption. For gel formation the removal of some if not all of the adsorbed ions is required.

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CHEMICAL STUDIES ON APPALACHIAN UPLAND PODSOL SOILS

I. CONDITIONS GOVERNING BASE-EXCHANGE RELATIONS[1]

By G. T. Shaw[2] and R. R. McKibbin[3]

Abstract

The base-exchange properties and the constituent materials of the base-exchange complexes of Quebec Appalachian upland podsol soils have been studied. It has been shown that the "availability" of the acid semihumified organic matter has played the most important part in bringing about existing conditions in these soils. Organic matter is dominating the processes through which these podsol soils pass in their progress from a slightly leached to a severely leached condition. The inorganic base-exchange complexes are superseded by organic complexes. The more "available" iron and aluminium present in these soils the less is their base-exchange capacity. The inorganic base-exchange complexes are unstable under the strongly acidic soil conditions. The restoration of fertility to these soils must be approached through modification of their organic-matter conditions.

Investigational work ((8) and Table I) has demonstrated the high degree of "availability" of a certain fraction of the organic matter and of the sesquioxides of iron and aluminium of podsol soils of the type described in this paper. Alkali treatment in small quantities effects instant separation of these fractions. The extreme ease with which some of the iron and aluminium can be removed may be further shown by leaching with $N/2$ acetic acid (Table II). This treatment extracts surprisingly large amounts of "soluble" or "available" sesquioxides. These experiments indicate the availability of these substances, suggesting the probability that in part they exist as a coating over the soil particles and so impress some characteristics on the soil as a whole. These conditions must be linked up with the processes at work in the formation of a podsol soil. Here the organic matter is believed to be primarily responsible for the production of the acidic environment in the soil. The "available" emulsoidal fraction acts as a protective agent in the removal of colloidal matter and sesquioxides of iron and aluminium to the lower horizons. There is close association of the organic matter with these "available" sesquioxides and with the transformations that a podsol soil undergoes as the forces of podsolization proceed. All of these substances owe their "availability" and their influence on soil conditions to their attachment to the surface of the soil particles. This is particularly true of the organic fraction. As will be indicated later, surface phenomena are predominant in these soils. Only in this way may the acidic condition characteristic of podsol soils be readily explained. We have thus a picture of the

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TABLE I

THE EFFECT OF SMALL ALKALI TREATMENTS IN LIBERATING THE ORGANIC MATTER OF APPALACHIAN UPLAND PODSOL SOILS

Chemical added to 1 kgm. of soil plus 25% moisture in each case	pH value	Total solids per litre, in gm.	Organic matter in solution per litre, in gm.	Chemical added to 1 kgm. of soil plus 25% moisture in each case	pH value	Total solids per litre, in gm.	Organic matter in solution per litre, in gm.
1. NaOH — 1 gm.	6.2	0.67	0.43	4. Untreated	5.5	0.12	0.07
2. Na_2CO_3 — 1 gm.	6.1	0.36	0.22	5. CaO — Equivalent to 1 gm. NaOH	6.4	0.24	0.11
3. KOH — Equivalent to 1 gm. NaOH	5.8	0.32	0.17	6. $CaCO_3$ — Equivalent to 1 gm. Na_2CO_3	6.4	0.12	0.06

NOTE:—*Distilled water was leached through the treated soils in the percolators after four weeks. The first litre was collected and analyzed, and in this the soluble carbonates exerted a misleading effect. A second litre, collected after seven weeks, was analyzed and the results in Table I obtained. Several soils were examined under each treatment and the figures shown are typical.*

TABLE II

THE SOLUBLE IRON AND ALUMINIUM LIBERATED FROM 100 GM. OF SOIL (CALCULATED ON MOISTURE-FREE BASIS) BY SUCCESSIVE LEACHINGS WITH 500 CC. OF *N*/2 ACETIC ACID

Soil	Fe_2O_3		Al_2O_3	
	Gm.	P.p.m.	Gm.	P.p.m.
1. 1st leaching	0.0007	7	0.0114	114
2nd leaching	0.0008	8	0.0122	122
2. 1st leaching	0.0033	33	0.0689	689
2nd leaching	0.0048	48	0.0521	521

NOTE:—*Burgess (1) mentions that soils of pH 5-5.8 usually have about 26 p.p.m. of soluble alumina. He used a more drastic method than the writers'; he shook the soils and acid together, and let the mixture stand, while in this work immediate leaching was used.*

surface action of this organic-matter fraction and a possible explanation of the consequent ease of its liberation by mild alkali treatment.

The technique employed in these studies was based on the method of Schollenberger (7), using neutral normal ammonium acetate as the agent for the removal of the exchangeable cations. A closed syphon system was used for the leaching operation. The individual basic cations were determined in the leachate after the replaceable hydrogen was determined by potentiometric back titration to neutrality. The total base-exchange capacity was found by determining the ammonium adsorbed in the soil after leaching.

Two cultivated podsol soils, representing the surface eight inches, were first analyzed for their different replaceable cations. Both soils were found to be deficient in the amounts of the individual basic cations Ca^{++}, Mg^{++}, Na^{+} and K^{+}. Highly fertile soils are known to possess quantities several

times the figures obtained. Podsolized soils are marked by this deficiency of exchangeable basic cations which reflects the low fertility of these soils.

The availability of the sesquioxides of iron and aluminium suggested the possibility that they might be present in some quantity in the base-exchange system, as other workers (2) have found when working with other types of soils. If they occurred to such an extent in these podsol soils, the fertility of the latter would probably be injuriously affected, as soluble iron and aluminium are considered to be deleterious to plant growth. However, they were found to be present to a very limited degree and it is doubtful if these traces of iron and aluminium sesquioxides really did exist in the exchangeable form. Undoubtedly they were released because of their association with the surface of the soil particles and were washed out by the leaching agent as it percolated through the soil. In these small traces they would not be able to affect soil fertility harmfully (see Table III). It is significant that the soil with more soluble iron and aluminium present has the lesser base-exchange capacity.

Another noticeable feature was the high amount of exchangeable hydrogen. In the two soils reported on, the hydrogen ions occupied 66 and 69% respectively of the total base-exchange capacity. This is termed "percentage unsaturation". The base-exchange system is quite obviously acting as a reservoir for hydrogen ions instead of being able to supply that reserve of basic cations which is so essential for fertility. A soil that has a large amount of exchangeable hydrogen soon loses a large part of its inorganic exchange complexes through disintegration into the constituent oxides of iron, aluminium and silica. In this way these constituents are converted into an available or free form and are characteristic of podsolized soil conditions. It is also poorly buffered to resist further increases in acidity through the action of additional sour organic-matter accumulation.

The picture drawn from these preliminary analyses reflects degraded soil conditions.

TABLE III

BASE EXCHANGE DATA FOR SURFACE CULTIVATED APPALACHIAN UPLAND PODSOL SOILS

Expressed as milli-equivalents per 100 gm. soil

Soil	Total capacity	Ca	Mg	Na	K	Al	Fe	H	% Base unsaturation	pH of air-dried soil	Total bases, m.e.
1	20.74	2.81	1.68	0.29	0.38	0.025	0.06	13.75	66	4.97	5.16
2	14.86	1.57	0.36	0.23	0.72	0.05	0.05	10.20	69	4.89	2.88

The analysis of the base-exchange relations of the subsoils, representing the soil lying between 8 and 18 in. in depth, again only emphasizes the low fertility from the base exchange standpoint. The "percentage unsaturation" was about identical with the corresponding surface soil layer. This indicates the highly buffered state of the base-exchange system — the fact that it retains its acidity even in the subsoil although there is a decrease in the amounts of organic matter and of replaceable hydrogen ions in the subsoil as compared to the surface layer (see Table IV).

TABLE IV

BASE-EXCHANGE DATA ON SUBSOILS OF CULTIVATED APPALACHIAN UPLAND PODSOL SOILS*

Soil	Ca	H	Total base-exchange capacity	% Unsaturation
1	2.73	9.15	15.27	60
2	2.56	3.91	6.24	63

**Expressed as milli-equivalents per 100 gm. of soil.*

In order to be able to draw further conclusions about base-exchange relations, it was necessary to base them on determinations on a number of representative upland podsol soils. Eight surface soils, sampled from the surface eight inches of virgin soil, and their corresponding subsoils, sampled from the soil between 16 and 24 in. in depth, were analyzed for replaceable H^+, Ca^{++}, Mg^{++} and total capacity. Sodium and potassium were not determined as they are generally present in very limited quantities. The procedure for these determinations was identical with that described previously.

Table V shows the results of these analyses. It may be seen that there is a large variation in the total capacities of the different soil samples. The basic cations, Ca^{++} and Mg^{++}, show similar wide variations. In most cases the hydrogen ions constitute a strikingly large proportion of the total replaceable ions as shown by the figures for the "percentage unsaturation" (given by dividing the replaceable hydrogen by the sum of the replaceable Ca^{++}, Mg^{++} and H^+, assuming that the sodium and potassium are present in unappreciable amounts so that they could be neglected).

TABLE V

REPLACEABLE CATIONS*

Soil no.	Surface soil						Subsoil					
	Ca	Mg	H	Sum	% Unsaturation	Measured capacity for base exchange	Ca	Mg	H	Sum	% Unsaturation	Measured capacity for base exchange
55	10.9	1.9	19.6	32.4	61	25.0	1.5	0.8	17.2	19.5	88	14.1
4	12.0	5.0	25.0	42.0	60	36.1	2.5	1.4	6.3	10.2	62	6.1
25	6.4	1.0	22.4	29.8	75	34.5	0.7	0.5	9.4	10.6	89	8.4
21	2.5	0.8	17.8	21.1	84	19.6	0.8	0.4	15.2	16.4	93	11.0
52	4.0	1.1	30.5	35.6	86	39.3	0.6	0.5	11.4	12.5	91	9.8
26	5.7	2.2	14.1	22.0	64	17.9	2.0	2.2	6.3	10.5	60	6.4
61	3.3	0.7	16.6	20.6	81	27.9	1.3	0.4	7.2	8.9	81	7.0
27	7.7	0.8	8.0	16.5	49	13.4	3.8	0.6	4.6	9.0	51	6.1

**Expressed as milli-equivalents per 100 gm. of moisture-free soil.*

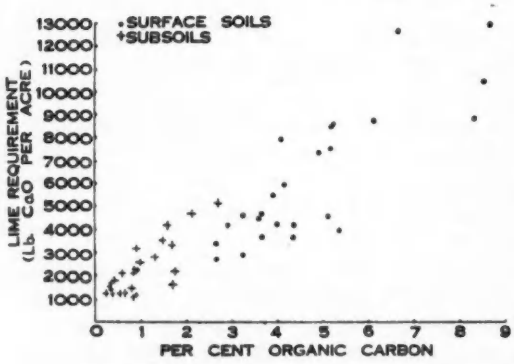

FIG. 1. *Relation between lime requirement and percentage organic carbon of soils and subsoils of virgin Appalachian upland podsol soils.*

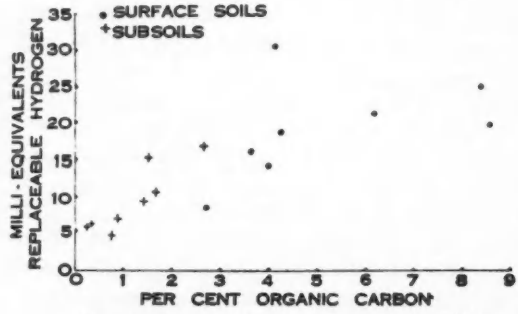

FIG. 2. *Relation between the replaceable hydrogen and the percentage organic carbon of surface soils and subsoils of virgin Appalachian upland podsol soils.*

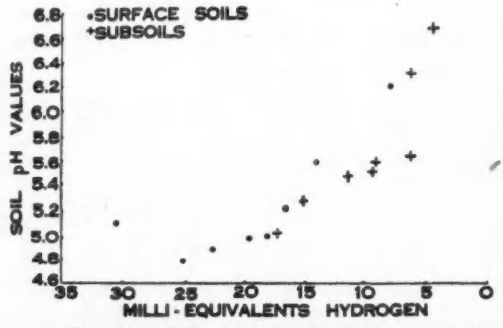

FIG. 3. *Relation between soil pH values and the replaceable hydrogen of surface soils and subsoils of virgin Appalachian upland podsol soils.*

It is recognized that the lime requirement is a measure of the "unsaturation" of a soil. This "unsaturation" is directly related to the acid conditions existing in the soil and may be said to be caused by the accumulation of sour semi-decomposed organic matter. The predominating influence of organic matter in an Appalachian upland podsol soil may be established by showing its relation to the lime requirement. By plotting the carbon content of each of 25 analyzed samples of virgin upland podsol soils against their corresponding lime requirement values, good agreement is obtained for the direct variation of the lime requirement with the percentage of carbon. This relation holds for both surface soils and subsoils ((4) and Fig. 1). The correlation is very good and holds for all virgin soils of this type taken from this area and analyzed (5, 6).

By plotting the replaceable hydrogen of the surface soils and subsoils against their percentage of organic carbon a direct relation is indicated (Fig. 2); the replaceable hydrogen increases directly as the organic matter increases. From the evidence presented in Figs. 1 and 2 it may be stated that the soil acidity is closely associated with the organic matter and probably exists in the organic exchange complexes to a great degree.

There is also a coincidence of degree of leaching with these factors, as measured by the thickness of the leached soil layer, and as given in Table VI. This is also indicated by plotting the soil pH values against the replaceable hydrogen, a direct line relation being obtained (see Fig. 3).

TABLE VI

ANALYTICAL DATA ON VIRGIN SOILS STUDIED IN BASE EXCHANGE WORK

Soil no.	pH value	Hygroscopic moisture, %	N, %	Organic carbon, %	C/N ratio	Thickness of A_2 horizon (leached layer), in.	Degree of leaching
55 Surface	4.90	8.24	0.40	8.52	21.2	1 — 3	Very heavy
Subsoil	5.13	4.00	0.13	2.68	21.2		
4 Surface	4.81	5.26	0.36	8.32	23.1	1 — 2	Very heavy
Subsoil	5.66	0.80	0.05	0.30	6.0		
25 Surface	4.88	4.86	0.07	6.12	87.9	1	Very heavy
Subsoil	5.49	2.15	0.03	1.28	45.7		
21 Surface	5.08	3.29	0.30	4.17	13.5	½ — 1	Heavy
Subsoil	5.31	2.01	0.07	1.48	20.2		
52 Surface	5.12	4.33	0.25	4.09	16.6	2 — 5	Very heavy
Subsoil	5.47	2.59	0.09	1.70	17.9		
26 Surface	5.59	2.53	0.12	4.00	32.6	½ — 1½	Heavy
Subsoil	6.30	1.12	0.05	0.23	4.6		
61 Surface	5.25	2.86	0.26	3.68	13.8	½ — 1	Light
Subsoil	5.59	1.74	0.06	0.92	14.6		
27 Surface	6.21	2.46	0.26	2.71	10.6	0 — ½	Very light
Subsoil	6.67	1.35	0.04	0.79	18.8		

The most significant relation is the direct variation of the total exchange capacity with organic carbon content. This is plotted in Fig. 4. As the percentage of carbon increases, the adsorption capacity of both surface soils and subsoils increases. It may be concluded that the exchange system is chiefly in the organic colloidal fraction rather than in the clay fraction. The organic complexes become more dominant as the organic matter accumulates and the inorganic complexes disintegrate.

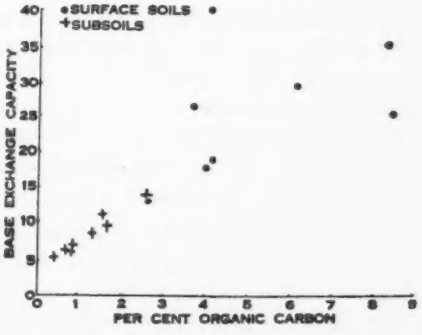

FIG. 4. *Relation between the base-exchange capacity and the percentage organic carbon of surface soils and subsoils of virgin Appalachian upland podsol soils.*

These deductions are also maintained by plotting the base-exchange capacity figures against the percentage hygroscopic moisture of the soils. The curve, showing that one value is a function of the other, is given in Fig. 5. The percentage of hygroscopic moisture can be taken as a relative measure of the organic colloidal fraction of these soils, the seat of the base-exchange complexes.

The phenomenon of organic base exchange has received little consideration and not much is definitely known about it. In the inorganic complexes the exchange cations are chemically united to the suspensoidal crystalline "acidoid" complex. In contrast, many organic colloids of the soil are emulsoidal. Organic matter for a given weight has a greater exchange capacity than the clay fraction. However, there is uncertainty as to how organic matter may function as a seat for replaceable ions and how they are adsorbed. It is important to note that acidity, which is not conducive to the stability of the inorganic complexes, does not act similarly on the organic exchange system of these podsol soils. The exchange capacity increases as the acidity increases instead of decreasing as might be expected. Thus it may be deduced that the base-exchange system does not exist in the inorganic fraction to any great degree. Organic complexes seem to function as an adsorption system in highly acid surroundings in contrast to the instability exhibited by inorganic complexes under these conditions. Evidently the inorganic complexes once existed in these soils to a larger extent but through acidic organic matter accumulation they are disintegrating and their influence is disappearing. As podsolization progresses they are being continually supplanted by the organic complexes.

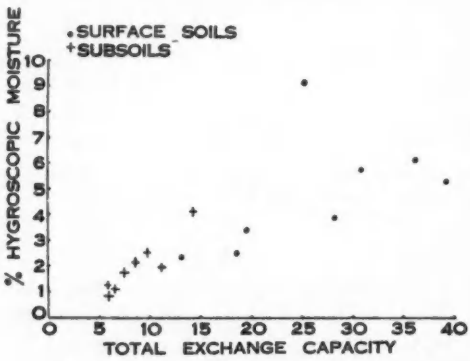

FIG. 5. *Relation between the percentage hygroscopic moisture and the total exchange capacity of the surface soils and subsoils of virgin Appalachian upland podsol soils.*

McGeorge (3) has shown that the lignin, ligno-hemicelluloses and lignocelluloses or related bodies function as the base-exchange complexes of soil organic matter. The writers' findings indicate that some relation may exist between the percentage nitrogen and the base-exchange capacity of these podsol soils (see Table VI and Fig. 6).

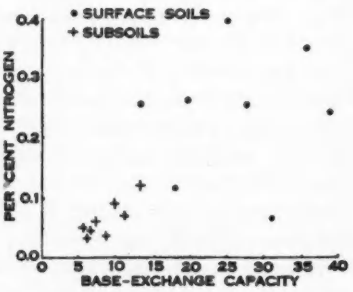

FIG. 6. *Relation between the percentage nitrogen and the total replaceable capacity of the surface soils and subsoils of virgin Appalachian upland podsol soils.*

No continuous relation is apparent when the amounts of replaceable calcium are plotted against the total exchange capacity. However, by plotting the replaceable calcium against the "percentage unsaturation" an interesting curve is produced. Points for individual soils are joined in a continuous line, according to the degree of leaching and the percentage of organic carbon in the soils. This is shown in Fig. 7. The upper line is for the surface soils as numbered, the lower one for the subsoils. It may be seen that the shapes of the two curves are similar and that the orders of the soils in the two curves approximately coincide. These curves illustrate that when there is either a relatively low or a relatively high amount of organic matter in an Appalachian upland podsol soil the "percentage unsaturation" is lower than it is in the case of the soils with an intermediate amount of organic matter (see Table VI). They indicate also that soils with a low "percentage unsaturation" possess a relatively high amount of replaceable calcium, although the soil concerned may have either a high or a low accumulation of organic matter. Soils with intermediate amounts of accumulated organic matter, having high "percentage unsaturation", contain relatively low amounts of replaceable calcium. The order of these soils in the curves also coincides with the degree of leaching (see Table VI), which in these soils is of course closely related to the amount of organic matter accumulation (4, 6).

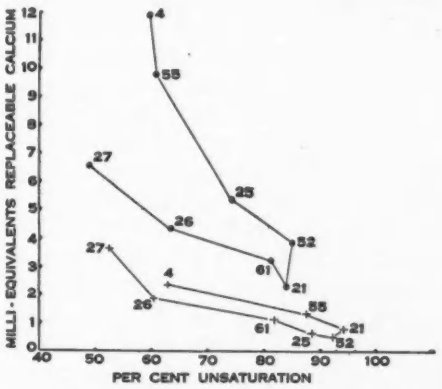

FIG. 7. *The replaceable calcium is plotted against the percentage unsaturation of surface soils (upper curve) and of subsoils (lower curve) of virgin Appalachian upland podsol soils.*

It may be inferred that those Appalachian upland podsol soils which have a low amount of organic matter and that are slightly podsolized have a relatively high proportion of their base-exchange systems in the inorganic clay complexes. With an increase in organic matter, podsolization increases and the soils become more acid. Thus the soils lose their lime reserves and the base-exchange complexes lose most of their basic cations, reaching a high "percentage unsaturation" as the exchangeable basic cations are replaced by the intensely active hydrogen ions.

This condition tends to produce the previously mentioned disintegration of adsorption complexes into their constituent oxides of iron, aluminium and silicon. Being freed, the sesquioxides are available for transportation to lower soil horizons through the solvent and protective action of certain fractions of the organic matter. The organic matter becomes, to a progressively greater extent, the seat of the adsorption complexes, replacing the destroyed inorganic complexes. As the new organic complexes form, the

exchange capacity increases. In time the greater portion of the adsorption system is situated in the organic fraction, with the soil becoming still more acid and podsolization more intensified due to the organic accumulations.

Unlike the inorganic complexes the organic complexes seem able to survive this condition of strong acidity for a long time. As accumulation of organic matter proceeds the organic base-exchange complexes appear to become better supplied with basic cations and have a slightly lower "percentage unsaturation". With increased base-exchange capacity in the organic fraction of the soil, basic cations may be better held and their leaching away considerably diminished. In soils Nos. 4, 55 and 25, for example, there are higher amounts of replaceable calcium, although these soils are heavily podsolized.

Some of the stages in the development of a podsolized soil may be explained by this picture. It should be noted that under the conditions in which these soils occur the lightly podsolized soils and those very heavily podsolized are more fertile than the others. The soils in an intermediate stage of organic matter accumulation are highly unsaturated in both surface and subsoil layers, which indicates that they have not sufficient exchangeable basic cations to aid in proper plant nutrition.

From the curve in Fig. 7 for the subsoils much the same conclusions may be reached. Here the conditions and influences producing alterations are not so intense as they are in the surface layer, so that the results are not so greatly divergent in soils which are existing at different degrees of podsolization. It will be noted that the curve for the subsoils is not spread out to the same extent as that representing conditions in the surface horizons. The same type of curve is given by replaceable magnesium.

In virgin podsol soils the surface horizon is the seat of the accumulated organic matter which is the cause of the processes active in podsolizing a soil. From this centre the activity associated with the effect of the organic matter commences to function and leaching occurs. Soluble electrolytes are gradually washed down and iron and aluminium are carried below. These influences remove the "available" minerals, and organic matter forms a coating over the soil mineral particles. The inorganic exchange system is supplanted by an organic one. The replaceable mineral or basic cations are largely displaced by the non-metallic hydrogen ion. In the manner described the upper horizons of a podsol soil are transformed from a mineral system to an organic one. According to this viewpoint podsolization is a demineralization process—the dominance of, and the effect of, certain fractions of organic matter on the reactivity of a soil. In the bleached horizon, the true "podsol" horizon, little colloidal material remains, either of an organic or inorganic nature.

From these data and from this discussion it may be seen that organic matter is believed to play a leading role in these podsol soils. It is the key through which their restoration to a more fertile plane must be attempted. The present state of the organic matter appears for many crops to be positively

harmful and it requires modification before the soils can be expected to become more fertile. Desirable alteration of its physical and chemical condition naturally aids in alleviation of the high lime requirement.

It is believed that any specific treatment may not be applicable to all upland podsol soils in attempting to increase their fertility. Soils only slightly podsolized and those in an advanced state of organic matter accumulation have been shown to be fairly well supplied with replaceable bases that are essential for plant growth. Such soils apparently need not be treated for their restoration in the same drastic manner as those in an intermediate podsolized condition. A modified treatment is required to suit the degree of podsolization. Soils which have attained relative "podsolic" maturity may be considered to have become dominated by the influences causing demineralization and coating of the mineral particles in the surface horizon by the organic film. In an immature podsol soil of the type discussed here transformational influences do not completely predominate and the soil retains its mineralized condition to a greater extent. Thus, it will be readily understood that the stage of development of podsolization will have an important bearing on whatever remedial applications are made to these soils.

These studies of base-exchange conditions illuminate the significant role of the acid organic matter accumulation in lowering the fertility of these Appalachian upland podsol soils.

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THE DENSITIES AND PARACHORS OF VINYL ACETATE AND ITS LIQUID POLYMERS[1]

By Charles Green[2], James Marsden[3], and A. C. Cuthbertson[4]

Abstract

The density of monomeric vinyl acetate has been determined over a range of temperature. The parachor and Ramsay and Shield's constant have been obtained for the monomer and some liquid polymers. On the assumption that a liquid polymer may be considered as a solution of a dimer in the monomer, parachors were calculated from the mixture law.

If Staudinger's formula be assumed for the dimer the calculated and observed parachors agree to within 1%.

In many cases polymerization is characterized by the repeated addition of a structural unit, and the substance when liquid passes successively through stages of increasing viscosity and eventually to the solid state.

In the course of an investigation of the polymerization of vinyl acetate some additive and constitutive property might in the authors' opinion serve as a guide to the mechanism of such a reaction in its initial stages. As Sugden's (5) parachor is both an additive and constitutive property of liquids the value of this quantity was determined during the initial stages of the polymerization of vinyl acetate. This substance was chosen because the degree to which it polymerizes is readily controlled and the available evidence points to a chain type of reaction. The density of vinyl acetate was determined over a temperature range.

The Density of Vinyl Acetate

The vinyl acetate* was distilled through a long spiral column to remove traces of acetaldehyde, acetic acid and copper acetate present as an inhibitor. The density measurements were carried out in a dilatometer. The values obtained are given in Table I.

TABLE I

Density measurements of vinyl acetate

Temp., °C.	Density, D_{4}^{t}	Temp., °C.	Density, D_{4}^{t}	Temp., °C.	Density, D_{4}^{t}	Temp., °C.	Density, D_{4}^{t}
9.0	0.9459	16.65	0.9360	20.00	0.9312	25.00	0.9243
10.40	0.9441	17.00	0.9355	21.00	0.9300	25.75	0.9235
12.50	0.9413	18.40	0.9336	22.00	0.9288	30.00	0.9181
15.00	0.9381	19.00	0.9327	24.00	0.9260	31.50	0.9161

[1] *Manuscript received May 11, 1933.*

Contribution from the Department of Chemistry, Mount Allison University, Sackville, New Brunswick, Canada. Constructed from a thesis presented by J. M. in partial fulfilment of the requirements for the degree of B.Sc.

[2] *Assistant in chemistry, Mount Allison University.*

[3] *Student, Mount Allison University.*

[4] *Assistant Professor of Chemistry, Mount Allison University.*

* *Kindly supplied by the Shawinigan Chemical Co.*

Within the accuracy of the results, 1 in 5000, the relation between density and temperature is linear. The equation $D_{4^\circ}^{t^\circ} = 0.9446 - 0.001326\ (t-10)$ represents the above data, where $D_{4^\circ}^{t^\circ}$ = density, and t = temperature in °C.

The Parachor of Vinyl Acetate and its Liquid Polymers

According to Sugden (5) the parachor is given by

$$P = \frac{M(y)^{\frac{1}{4}}}{D-d},$$

where P = parachor of a substance, y = surface tension, M = molecular weight, D = density of the liquid, and d = density of the vapor. Usually d is neglected so that the expression becomes $P = \dfrac{M(y)^{\frac{1}{4}}}{D}$.

Surface Tension Measurements

The surface tension was measured by the capillary rise method as approved by Richards (3). A fine capillary tube was sealed to a large tube, 4 cm. in diameter, bent in the form of a U, so that the planes were as nearly parallel as possible. The capillary was chosen from a number for its regularity in bore and because its cross section was almost a perfect circle.

The Radius of the Capillary

The radius was determined by measuring the length of a column of mercury in the capillary with a travelling microscope. A correction was made for the protruding meniscus according to Winkler (6) and also on the assumption that for small capillaries the meniscus was a hemisphere. From two determinations the radius by the latter method was 0.02555 cm., by the former 0.02552 cm. Using the value of 0.02555 cm. the surface tension of water at 25°C. was found to be 71.70 dynes per cm., in good agreement with the accepted value 71.90.

Molecular Weight Determinations

The molecular weights of the polymerized products were determined by the conventional Beckmann freezing point method, using benzene as solvent. The effect of the solvent was investigated by using nitrobenzene and comparing the values for the molecular weights obtained. This was done for the monomer. No anomalous behavior was noted. The polymers are either insoluble or difficultly soluble in polar solvents.

The Densities

The densities were measured in the same dilatometer which had been calibrated for the determination of the density of the monomeric vinyl acetate previously mentioned.

The Preparation of the Samples of Vinyl Acetate

After distillation of the vinyl acetate in air, about 15 cc. was placed in each of five test tubes which had been previously cleaned in chromic acid solution, washed with distilled water and dried in air. These tubes were sealed off, following which they were exposed to light and heat from tungsten lamps enclosed in a reflecting tin box. The temperature was about 137° C. A sample was removed every two hours.

The results for surface tension, molecular weights and densities of the six samples are given in Table II. The parachor and Ramsay and Shield's constant are shown in Table III.

TABLE II

SURFACE TENSION, MOLECULAR WEIGHTS AND DENSITIES OF SIX SAMPLES OF VINYL ACETATE

Sample	Temp., °C.	Surface tension, dynes per cm.	Density	Wt. of vinyl acetate	Wt. of benzene	Temp. drop	Mol. wt.
I	20	23.95	0.9312				
	25	23.16	0.9243				
	30	22.54	0.9181	0.1716	15.806	0.637	86.38
II	20	23.89	0.9548				
	25	23.48	0.9482				
	30	22.81	0.9417	0.1371	16.420	0.453	94.39
III	20	24.15	0.9603				
	25	23.53	0.9536				
	30	.....	0.9471	0.1371	17.592	0.409	97.57
IV	20	24.10	0.9638				
	25	23.55	0.9573				
	30	22.91	0.9509	0.1196	17.159	0.365	97.74
V	20	24.31	0.9709				
	25	23.70	0.9634				
	30	23.01	0.9571	0.1258	17.491	0.360	102.3
VI	20	.....	0.9716				
	25	23.61	0.9648				
	30	.....	0.9581	0.1234	17.570	0.326	110.4

TABLE III

THE PARACHOR AND RAMSAY AND SHIELD'S CONSTANT FOR SAMPLES OF VINYL ACETATE

Sample	Parachor	Ramsay and Shield's const. 20–30°C.	Sample	Parachor	Ramsay and Shield's const. 20–30°C.
I	204.5		IV	224.8	
	205.0			225.0	
	204.5	—2.41		224.9	—2.14*
II	218.5		V	234.0	
	219.2			234.4	
	219.1	—1.88		234.2	—2.38
III	225.2	20–25°C.	VI	252.2	
	225.3	—2.22			

Theoretical

It is assumed that few if any of the molecular species present in the vinyl acetate at the stages of polymerization considered are higher than dimers. On this basis the degree of polymerization may be calculated from the following formula,

$$\alpha = \frac{M_o - M_t}{M_o(1-1/n)}$$

where α = degree of polymerization, M_o = observed molecular weight, M_t = molecular weight of monomer = 86.05, and $n = 2$.

The degree of polymerization of the samples has been calculated and is shown in Table IV.

TABLE IV

POLYMERIZATION OF VINYL ACETATE

Sample	I	II	III	IV	V	VI
Degree of polymerization	0	0.177	0.236	0.239	0.318	0.441

Assuming the presence of a dimer of molecular weight $2 \times 86.05 = 172.1$ the mole fraction of the dimer may be calculated in each case, *i.e.*, mole fraction of the dimer in II

$$= \frac{\frac{0.177}{172.1}}{\frac{0.177}{172.1} + \frac{0.823}{86.05}}$$

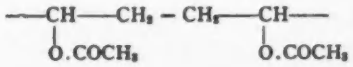
The mole fractions are given in Table V.

TABLE V

THE MOLE FRACTIONS OF MONOMER AND DIMER IN THE VARIOUS SAMPLES

Sample	Mole fraction dimer	Mole fraction monomer	Sample	Mole fraction dimer	Mole fraction monomer
I	0.	1.000	IV	0.136	0.864
II	0.097	0.903	V	0.189	0.811
III	0.134	0.866	VI	0.283	0.717

Staudinger (4) has suggested that vinyl acetate polymerizes according to the following scheme



If it be assumed that vinyl acetate, polymerized to the states in question, is a mixture of the monomer and dimer it was interesting to find whether for a given sample, the mole fraction of the dimer × parachor of the dimer + the mole fraction of the monomer × parachor of the monomer = the observed parachor as determined experimentally. Taking the atomic values*, C = 4.8, H = 17.1, O_2 in esters = 60; the parachor for the dimer = 363.6.

Table VI shows the observed and calculated values of the parachors of the six samples.

TABLE VI

THE OBSERVED AND CALCULATED VALUES OF THE PARACHORS OF THE SIX SAMPLES

Sample No.	Parachor		Diff., calcd.–obs.	Sample No.	Parachor		Diff., calcd.–obs.
	Obs.	Calcd.			Obs.	Calcd.	
I	204.7	205.0	0.3	IV	224.9	226.5	1.7
II	218.9	220.4	1.5	V	234.2	235.0	0.8
III	225.3	226.7	1.4	VI	252.2	249.9	−2.3

Discussion

In the calculations referred to, the assumption has been made that the dimeric vinyl acetate is dissolved in the monomer. If x is the mole fraction of the dimer, P_x = parachor of the dimer, P = parachor of the monomer and P_m the parachor of the solution, $P_m = P(1-x) + P_x x$. Hammick and Andrew (1) have applied this equation to the determination of the parachors of organic liquids in polar and non-polar organic solvents. They found that the mixture law gives either the value of the parachor or a series of values from which the true value can be obtained by extrapolation.

The application of the mixture law to vinyl acetate is of course justified only in so far as the calculated and observed parachors of the liquid polymers agree. A certain difficulty arises in the measurement of the surface tension of viscous solutions. This difficulty might be overcome by dissolving the polymer in benzene and applying the mixture law to the solution obtained.

The value for the Ramsay and Shield's constant of the monomer is somewhat higher than the average value for a normal liquid. A number of acetates however give a value of 2.3, others as high as 2.6. The value of 2.4 is therefore in good agreement. Judging from the values of the constant the polymers act like associated liquids with the distinction probably that, while the phenomenon of association is reversible, polymerization is not.

*Mumford and Phillips' (2) values gave a calculated parachor of 201.2 for the monomer allowing for a value of −6 for strain constants.

Staudinger's (4) formula for vinyl acetate is based on the evidence that the decomposition product of the polymers is oxalic acid, and on the assumption that it is a chain reaction. The agreement between the calculated and observed values for the parachor confirm this formula.

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AN EXPERIMENTAL INVESTIGATION OF PROBABILITY[1]

By A. L. Clark[2]

Abstract

An experimental determination of probability is described in this paper. Steel balls were dropped on to a horizontal plate pierced with holes in regular array, and the number which passed through without contact with the plate was observed. The ratio of the number of free passages to the total number of balls dropped is the experimental probability, and this number was found to be in very close agreement with the probability calculated from measurement of the balls and holes. Since this calculated probability involves π, the experimental probability may be used to calculate π which was found to be $3.143 \pm .005$. The paper closes with a brief discussion of the application of probability to certain problems in physics.

The experiment described in the following paper was evolved as a laboratory experiment for third year honor students in physics. An experiment was sought which would combine freedom from monotony, easy discrimination between favorable and unfavorable cases and speed of operation and have the added interest of furnishing a method for deducing the value of the constant π.

The well-known problem of Buffon (2, p. 98) as the basis of an experiment was rejected because it lacks the first three desiderata although it contains the interesting feature of yielding the value of π.

It is perhaps unnecessary to say that a contact with probability through an experiment is highly desirable for students of physics and mathematics.

Steel balls are dropped vertically at random on to a horizontal steel plate which is bored with circular holes in hexagonal array and the observer records whether or not they pass through the holes without contact with the plate. The probability that a ball dropped at random will make a free passage is given by:

$$P = \frac{2\pi (R - r)^2}{d^2 \sqrt{3}} \tag{1}$$

for the hexagonal array, or would be

$$\frac{\pi (R - r)^2}{d^2}$$

for a square array. R and r are the radii of holes and balls respectively and d is the centre distance.

The hexagonal array was chosen because it gives a slightly larger P for the same distance d. It is desirable that the probability should not be small for then the interest would suffer. The distance d must not be too small compared with R, otherwise there would be a large distortion of the holes already bored when boring the adjacent holes.

[1] *Original manuscript received June 19, 1933.*
Contribution from the Department of Physics, Queen's University, Kingston, Ont., Canada. This paper was read at the May meeting of the Royal Society of Canada, 1933.
[2] *Dean of Applied Science, Queen's University.*

Apparatus

The plate (Fig. 1) is of steel about 1 ft. square and $\frac{1}{4}$ in. thick. It is suspended on supporting arms on a shallow frame $1\frac{1}{2}$ ft. square. This frame is closed on the lower side by a cloth shaped like an inverted tent which serves the double purpose of catching the balls without sound and delivering them to a small cup through a hole at the vertex of the cone. The plate may be moved six inches and is treated as though it were infinite in extent.

The dropper proper, shown in Fig. 2, consists of a trough which acts as a hopper and allows the balls to run freely into the vertical dropping tube. The lower end of this tube is closed by a sliding plate pierced with a $\frac{3}{16}$-in. hole. This plate is placed at the end of a flat spring which is pushed forward by a plunger operated by an electromagnet. Attached also to the spring is a tongue of metal which moves in between the lowest two balls when the plate moves forward to allow a ball to drop from the tube. Thus one and only one ball at a time is allowed to fall.

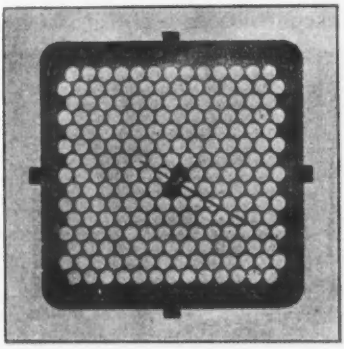

FIG 1. *The plate.*

The balls fall from the dropper into a shallow pan-shaped funnel in which they roll to the centre into the vertical delivery tube through which they fall on to the plate. This delivery tube with the attached pan is supported on horizontal rods which rest on a frame about three inches square. The delivery tube may be moved through a range of three inches and, no matter where the plate may be, is always over the holes in the plate.

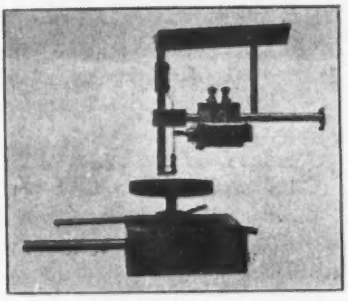

FIG. 2. *The dropper.*

The plate and delivery tube are moved after each ball is released and the double motion provides the randomness which motion of either alone might lack. The operator might fall into a routine with one motion only but is less likely to do so with the two. When the apparatus is used by students, a screen is placed in front of the delivery tube so that the person moving the plate does not see what the one moving the delivery tube has done. The results show that randomness, the essence of a probability experiment, has been achieved. Fig. 3 shows the assembled apparatus.

Fifty balls are placed in the hopper and released one by one. The record is made by indicating by symbols in the record book whether or not the ball touched the plate. A microphone is attached to the lower side of the plate and is in series with a single dry cell and a pair of ear-phones. This micro-

phone is the carbon capsule from an ordinary Northern Electric telephone transmitter. The slightest contact of a ball with the plate gives a decided noise in the phones. While there may be a slight doubt as to just what constitutes a contact, the region of uncertainty must be very small indeed. If a sound is heard, contact is assumed; if there is no sound, a free passage is recorded.

Observations may be made and recorded with great rapidity. Even inexperienced observers can digest the problem and record 500 trials in a two-hour laboratory period. The results obtained by each pair of students are recorded in a log of the experiment and the experimental values of the probability by 100's and for all observations to date are drawn day by day on a continuous graph.

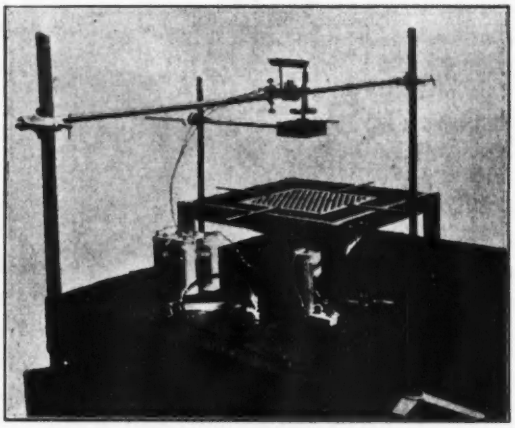

FIG. 3. *The assembled apparatus.*

The results which follow were obtained by various observers but largely by Mr. E. Harris, Curator in the Physics Department, whose interest and enthusiasm led him to carry the record to 100,000 events after the goal of 50,000 had been reached.

Measurements and Errors

The diameters of the balls were found by measuring many random samples. The accuracy with which these balls* are made is extraordinary. Not only do single balls vary but little but the variation of the diameters of different balls is almost nil. The diameters were measured with two micrometers, one a new metric instrument, the other an excellent shop micrometer graduated in inches. These were checked with Johannsen gauges. The diameter was found with a variation of not over 0.0001 and is taken as 0.1872 in.

The diameters of the holes and the distances between centres were found by using two steel cylinders carefully made with an almost infinitesimal taper. These fit the holes snugly and are calipered for the diameter. The diameter of the holes is 0.6572 in. For the centre distance, the cylinders are pressed into adjacent holes and a tapering steel scale pushed gently between them. This scale is five inches long and is graduated into 50 parts. Each of these parts represents $\frac{1}{1000}$ in. so that readings may be made by estimation to 0.0001 in. The cylinders and scale are shown in position in Fig. 1.

* *The balls used are the regular Hoffman balls for bearings.*

The mean centre distance is 0.7507 in. for the horizontal lines of holes, and 0.7499 in. for the inclined lines. Since operations involving $\sqrt{3}$ are not contemplated in the construction of a milling machine it is not possible to get the two sets of distances alike. The results show also that the main screw of the milling machine is not quite exact. What was supposed to be 0.75 is 0.7507 in.

Since the two sets of centre distances vary, a better expression for the probability is

$$P = \frac{2\pi\,(R - r)^2}{a\sqrt{4b^2 - a^2}} \tag{2}$$

where a is the mean of the horizontal and b that of the inclined distance.

A source of error not foreseen comes from the fact that the balls do not fall quite vertically. If the plate were infinitely thin, this would not matter very much since the departure from the vertical is small. Looking along the direction of the tangent to the path where it meets the plate, one sees the upper circle of the hole as slightly elliptical, but sees also the wall of the hole. A ball moving along this direction may fall into the hole only to meet the wall. The effective area of the hole is reduced therefore by an amount depending on the amount that the path departs from the vertical.

It is possible to calculate in turn, the vertical component of the velocity, the horizontal component and the angle between the path and the normal to the plate. Then the reduction in area of the hole may be calculated for a series of distances from the centre line and a curve drawn. We have then the means of determining the effective area for any distance from the centre. A number of spotting runs of 50 each were made with carbon paper, the central point located for each with a plumb bob and a target diagram drawn. By counting the number of spots in each ring a fair estimate of the mean error may be found. A number of such tests yielded the mean factor 0.9983 which must be multiplied into the value of P calculated from the measurement. The error in this factor is less than those already discussed.

When all sources of error are taken into account the value of the probability of a free passage is

$$P = 0.3554 \pm .0005.$$

Results

According to our notions of probability, the larger the number of events, the more closely the experimental probability should approach the theoretical value. The experimental probability in any series is the ratio of the number of free passages to the total number of cases. Hence if the total number of events is continually increased, the graph of the experimental probability should approach the theoretical value or should approximate a straight line.

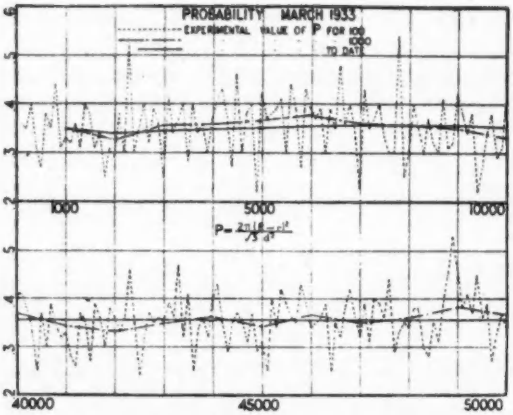

FIG. 4. *The values of the experimental probability by 100's.*

The diagram of Fig. 4 shows the values of the experimental probability by 100's, where the saw-shaped broken line shows the experimental values and the full line the cumulative value or the "value to date." Each point on this graph represents the value of the experimental P for all the observations taken to that point. The first and fifth tens of thousands only are given in order to show the straightening out of the graph.

Fig. 5 shows the same thing but plotted to a very much larger scale and by thousands instead of by hundreds. The graph of the value to date is carried to 100,000 and again straightens out very clearly. The terminal value cannot be far from the truth. The scale was again enlarged and the results plotted (Fig. 6) by 5000's and by 10,000's. The cumulative value calculated from the 5000 groups again shows the approach to a mean.

Since the result at the end of a series is not necessarily the best result, we may attempt to find the true mean by inserting a value between those that seem too high and those that seem too low. On the low-scale diagram this would show nothing, but on the large-scale diagram the method is fruitful. By this method we arrive at the value 0.35556 (see Fig. 6).

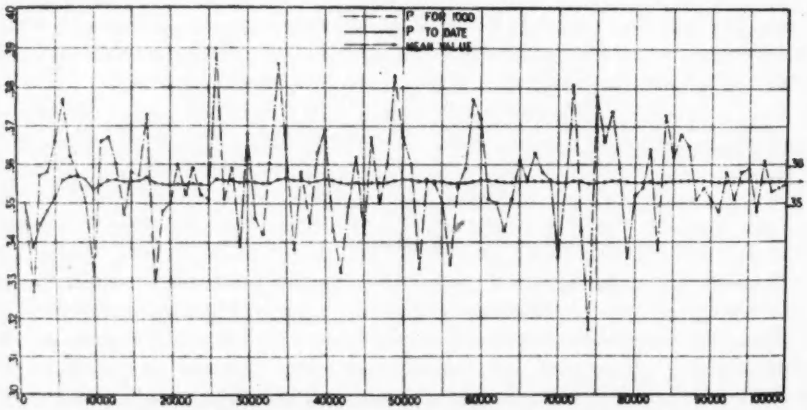

FIG. 5. *The values of the experimental probability by 1000's.*

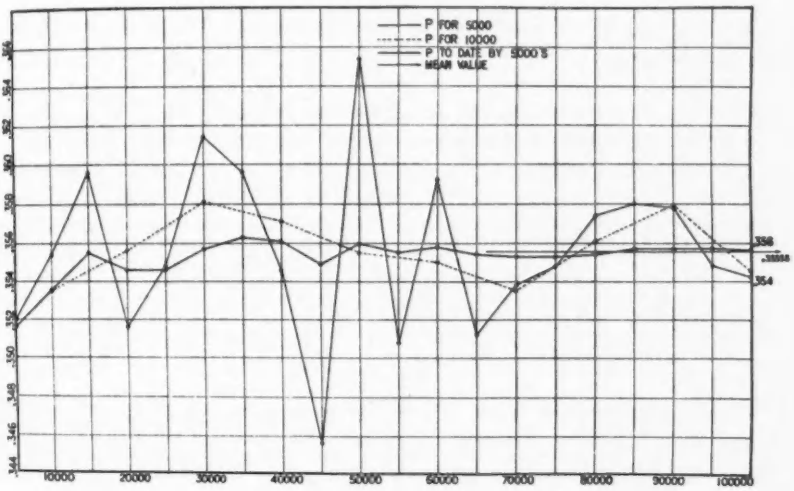

FIG. 6. *The values of the experimental probability by 5000's and 10000's.*

The terminal value is 0.35558. The value 0.35556 may be taken as a very close approximation indeed to the true value and is certainly more accurate than the calculated value.

The agreement between calculated and observed values of P, while gratifying is a little misleading. It is closer than the accuracy of measurement of the radii and centre distances might be expected to give and shows perhaps that the errors in these measurements partly neutralize each other.

If the experimental values of P by 100's are plotted against the number of times that each occurs we have a very good picture of the ordinary probability curve (Fig. 7). This curve also shows the fluctuations about the mean, taken here as 0.36. The small fluctuations appear often, the large seldom.

These fluctuations are very interesting. If we take extreme values of the number of free passages for groups of series of different lengths the graph of the fluctuations (Fig. 8) is of startling smoothness.

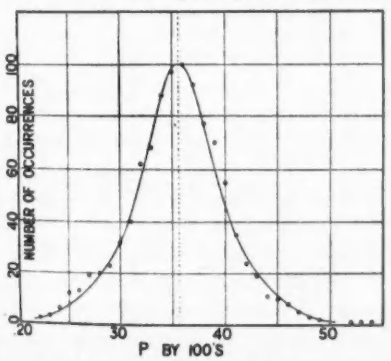

FIG. 7. *Distribution of values of P.*

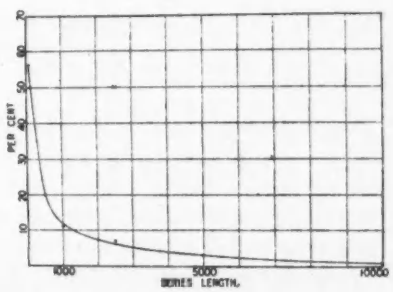

FIG. 8. *Per cent maximum fluctuations.*

Table I shows the values of P by 5000's and by 10,000's, and of course the fluctuations.

TABLE I

VALUES OF P BY 5000'S AND 10000'S

	By 5000's	By 10000's		By 5000's	By 10000's		By 5000's		By 5000's
1	0.3516	0.3535	6	0.3614	0.3550	11	0.3508	16	0.3574
2	0.3554	0.3556	7	0.3596	0.3525	12	0.3592	17	0.3580
3	0.3596	0.3581	8	0.3546	0.3561	13	0.3512	18	0.3578
4	0.3516	0.3571	9	0.3456	0.3579	14	0.3538	19	0.3548
5	0·3548	0.3555	10	0.3654	0.3545	15	0.3548	20	0.3542

Sequences

Finally and perhaps what is most important is the table of sequences (Table II). If P is the probability of a favorable event, P^2 is the probability that there will be two such events in succession, P^3 that there will be three,

TABLE II

SEQUENCES IN 100,000 EVENTS. $P = 0.3556$

	Runs calculated	Runs observed		Runs calculated	Runs observed
1's	35,560	35,558	8's	26	33
2's	12.676	12,259	9's	9	17
3's	4497	4655	10's	3	9
4's	1599	1489	11's	1	5
5's	569	572	12's	.4	3
6's	202	191	13's	.1	1
7's	72	74			

etc. Then if n is the total number of events, k the number in a sequence, the number of such sequences is

$$N = P^k(n - k + 1).$$

If n is very large compared with k this becomes

$$N = P^k n .$$

The table shows the calculated and observed values of N for 100,000 trials. Attention is called to the occurrence of sequences of 12 and 13 which appeared "out of due season." These are all the more important and interesting because of their early appearance and will be referred to later. Since a 13 counts as two 12's, three 11's, etc., the 12 and 13 observed make the numbers of 8's, 9's and 10's a little larger than they should be. The effect on shorter sequences is unimportant.

The Determination of π

Obviously if 0.3554, the calculated value of P, is substituted in (2) corrected, the value of π (3.1416) used in the calculation comes out. If 0.35556, the observed value of P, is used the value of π is slightly larger than the correct value, *viz.*, 3.143. This is the best value obtainable if the value of P is determined properly, *i.e.*, the result of a predetermined random series. If however, a value of P were to be selected from the observations a closer value of π might be obtained, indeed a very high degree of precision might result. The experiment however cannot be expected to give a value closer than 0.1% and any such selection is indefensible.

The celebrated problem of Buffon has been used by a number of observers to determine π. La Place seems to have been the first to suggest such a use. The problem may be stated thus. If a straight rod or needle of length c is cast at random on to a horizontal plane surface ruled with a series of straight parallel lines at a distance a apart, where $c>a$, what is the probability that the rod lies across one of the lines? As shown by Buffon the value is

$$P = \frac{2c}{\pi a} .$$

Wolf (2, p. 169) in 1850 using 5000 casts found the value of $\pi = 3.596$. Smith in 1855 with 3204 casts obtained 3.1553, Fox in 1894 with 1120 casts, 3.1419 and Lazzarini in 1901 with 3408 casts arrived at the astounding value 3.1415929.

All except Wolf seem to have selected the most favorable place to end the experiment. No such accuracy as indicated would be possible from a random series. The results therefore are not important.

The experiment of Buffon has a certain advantage over the experiment described in this paper. Only two measurements, a and c, are necessary and these measurements enter the calculation in the first power only, so that the errors are not magnified. In the ball experiment there are three measurements and they enter the problem in the second power so that the individual errors are doubled.

On the other hand there must be a number of cases where it is difficult to tell whether the end of the needle lies on a line or not. In the ball experiment, contact with the plate is easily determined if the microphone is used. While even then there must be a very small indefinite region where there is a little doubt about contact there is no doubt whatever about the response of the microphone. The noise, if any, is loud and unmistakable and there is no gradation of the sound.

It is probably as easy to caliper smooth cylindrical or spherical surfaces as to measure distances between such lines as would be used in an experiment. From the point of view of interest, the ball experiment is superior to that based on the problem of Buffon. There is a stimulation of interest in the waiting for the click and in the movement of the plate. The observations are made very rapidly and the monotony of the observation is not excessive. The balls are not returned to the hopper until fifty observations have been made.

General Remarks

While the results of experiments in probability prove very little, the conformity with theory exhibited in this experiment and the close approximation of the experimental to the calculated results are significant. And while one should not base arguments on these results alone, they suggest applications and lead naturally to the discussion of important points. Some of these points will now be taken up.

In the writings of certain authors, there is an implication that the extremely improbable in a series of events will happen if the series is sufficiently extended. Chwolson (1, p. 453), gives an example of this kind of statement. There, in what is one of the best treatments of the second law of thermodynamics the writer has seen, the idea of waiting for an improbable event which will happen if the time is long enough, is emphasized. The more improbable the event, the longer one must wait. As a matter of fact, the improbable may occur early in the course of events, or it may not occur at all. There is no reason why the improbable should be relegated to the distant part of a series of events or why it must certainly occur. One place in the series is as favorable as another.

The suggestion that if an improbable phenomenon has at any time not yet occurred, it is only necessary to wait for it to come later, is a trifle naive, and the explanation of its failure to occur may be given at a much later time with exactly the same assurance.

There is another kind of implication in writings of quite another sort, the so-called popular treatment of modern scientific discovery seen so often in the newspapers of our day. Such statements as "a kettle of water over a fire boils instead of freezes simply because boiling is more probable than freezing." One may infer that the improbable freezing might happen somewhere at some time. No one has ever heard of such a phenomenon nor does any one for a moment expect that it will ever happen. Such statements are misleading and the writer believes them untrue.

If we turn for a moment to the experimental work described in this paper, some light may appear. Consider first the fluctuations. If the experimental probability is calculated from groups of 50 observations each, the values lie between 8 and 28, *i.e.*, there are fluctuations about the mean, as large as 10, or the percentage fluctuation is 56. For groups of 100 the percentage is 50, for 500 it is 20, for 1,000 it is 11, etc. (Fig. 8).

As would be expected, the percentage or relative fluctuation falls off rapidly as the series length increases. For groups of 100,000 each we may predict a low value safely, for 1,000,000, a very low value and for extremely long series an almost vanishing relative fluctuation. This decrease in the value of the fluctuation about the mean is well known and the application to the explanation of the Brownian movement and other phenomena is familiar to students of physics. The numerical results derived from the data of the experiment are interesting however, and the ease with which the smooth curve of Fig. 8 was drawn is suggestive.

If we sample the results by selecting at random a number of groups of 50 consecutive events each, we find that the experimental probability, the fluctuations, the sequences and the distributions of favorable and unfavorable cases are very different. If however, we take samples of 1,000 each we find that in some respects these samples are very much alike, in others very different. The experimental probabilities and the extreme fluctuations are each very much the same and for any "run of mine" thousands will be remarkably like the sample. If we take samples of 10,000 each, this statement is much more forcible. If the experiment had been extended so that samples of 100,000 or more could be taken, it is almost certain that the variation in P and in the range of fluctuations would be found to be almost nil.

There are however important differences in the distribution of favorable and unfavorable cases even in long sequences. For example, in one place there appeared a sequence of 13 favorable cases, followed almost immediately by 21 unfavorable cases. Another small series had eight singles, another four four's, another, a long row of alternate favorable and unfavorable cases. These are not duplicated and no doubt there are many other oddities. Thus provided the series is fairly long, we may expect certain aspects of a series to change but little, but other aspects in which each may be unique. Of course it may be argued that if the observations are carried to very great numbers, all of these singularities or oddities will repeat. No one can refute such a statement but it is equally impossible to refute the statement that all of these particular ones will not repeat.

There is of course the most probable combination of hits and misses in, say, 50 events, and this might be found many times. The peculiarities referred to are far less probable. That there would be a sequence of 13 passages followed almost immediately by 21 misses was very improbable. Any particular distribution may have a probability attached to it and if the probability is small enough we might be tempted to say that the distribution will not occur. But perhaps it did occur! And perhaps the particular distribution referred to came early!

That a sequence of 12 or 13 should appear anywhere in the experiment was most unexpected. Indeed, according to theory a 13 might be expected once in a million events, yet it appeared in the 21st thousand. The 12 appeared in the 49th thousand. Either might have appeared anywhere else, might indeed have occurred in the same thousand. There is nothing to distinguish the thousands and neither time nor place in a series has anything to do with the matter.

Again where perfect randomness obtains, what has happened has no influence on what may happen. This is contrary to an instinctive feeling which is common to most people, even the initiated. For example, if a coin is tossed repeatedly and heads has appeared, say, five times in succession, the exercise of a rather strong will would be necessary to enable one to call "heads" for the next throw. However it is as likely then as it ever was or ever will be and the

probability is one-half.* The appearance of the 13 in the 19th thousand of the experiment described in this paper had no influence for or against the appearance of the 12 in the 49th thousand. The repetition of certain types of hands in cards and what is generally called a "run of luck" must be explained either as a result of lack of randomness or they may be just cases where improbable events appeared early in the series. (For modern bridge players they may not be very early.)

What about the appearance of the extremely improbable, say, a run of 1,000 in the experiment? The probability of such a sequence is 0.3556^{1000}, a very small number. Might it appear? This question is getting near the core of a very important matter, *i.e.*, the occurrence in nature of an event which is said to be extremely improbable, but is really treated as though it were impossible.

The mathematician would certainly answer in the affirmative. Assuredly, if there is a probability that a phenomenon may take place, the possibility of its occurrence must be admitted. Any one who has watched the course of the experiments of this paper, and has seen thousands of balls dropped, finds it difficult to entertain the possibility of such an excessively long run. Feeling however is a very poor guide in answering the question and if the mathematical theory may be applied to such extreme cases, the possibility of runs of any length must be granted.

A dart with a needle point thrown at random on to a large target must find a resting place which is so small compared with the available space as to give for this resting place an almost infinitesimal probability. Here apparently, the extremely improbable must happen at each cast of the dart.

Or turn to the tossing of a coin. Suppose a perfect coin to be tossed 100,000 times. The probability for heads at each cast being $\frac{1}{2}$, the total number of times that heads appears should be approximately 50,000. So much we may predict with confidence. What about the distribution of heads and tails? We know absolutely nothing about it, save that before any cast the probability for head or tail is always $\frac{1}{2}$. Since this is true for every cast any configuration of the series is as likely as any other and the probability of any particular configuration is a number so small as to be almost zero. But it may occur and one of these highly improbable configurations must occur, improbable as it is.

That the extremely improbable happens in every day life is well known to everyone. All can recall phenomena which before they appeared would have had very low probabilities.†

* *It is almost certain that prolonged experiments with dice and coins would show a slight predilection for one face. The construction of an ordinary die even if perfectly made must displace the centre of gravity toward the "one" and may make the "six" appear oftener than it should. The same kind of criticism may be offered regarding a coin experiment. If it is true in either case that the centre of gravity is displaced ever so little there will be a few critical cases in a long series where this displacement will determine the way in which the die or coin will lie.*

†*The writer well remembers standing as one of a group of small boys looking eagerly aloft to try to discern a small steel arrow that had been shot vertically upward and which had disappeared from sight. The first knowledge which any of the group had of the return was the sound of it plunging into the breast pocket of a member of the group. If the probability of that destination had been calculated it would have been extremely small.*

To return to the question. May the extremely improbable occur in nature and is there any limit to what may happen? If two bodies at different temperatures are placed in contact, the postulate underlying the second law of thermodynamics states that their temperatures will approach each other and ultimately will become equal. According to the work of Boltzman, Planck and others, processes of this kind go in a definite direction, always in the direction of greater probability, *i.e.*, the molecules interact in such a way that the final configuration is more probable than the initial. If it be a matter of probability only, is there the slightest hope that one might observe the warm body becoming warmer and the cold one colder?

Or is there a chance that all of the gas molecules in a small region in a child's balloon will move away from the wall at the same instant, causing a dimple to appear? Or does one expect to see the needle of a steam gauge move suddenly or a stone leap into the air? If two chambers containing different gases at the same temperature and pressure are put into communication, the gases mix. Will they ever unmix? Planck (3, p. 50) has discussed problems like some of these and has given the answer somewhat as follows.

Pure mechanical motions are reversible. The motions of individual atoms and molecules are pure mechanical motions and subject to dynamical laws. But the mixture of gases is highly reversible. Each individual particle acting on another performs pure mechanical motion and the motions of the pair are reversible. In the mass however the motions of a great number are irreversible. We are able to say nothing about individual molecules in a mass of gas. We cannot see the particles; we don't know where any one may go or what it may do. The limitation imposed on us because of our inability to deal with individual molecules drives us to probability methods. We say that the swarm of particles will go from a less probable to a more probable configuration and complete mixture is the result. We say that a reversal of the phenomenon is so improbable that it does not happen.

We may put a similar question. Why does entropy increase in natural processes and never decrease? The answer is exactly the same, only the question is put in a different form.

If we deal with two or three or any small number of particles, the differences between the probabilities of different configurations are small so that any possible configuration may appear and the actions of these particles are reversible. If two molecules say of nitrogen and two of oxygen mix without reaction, they may separate readily. When then does the reversible become irreversible? According to Planck, when the number of particles becomes large enough to yield a mean value of the variables such as pressure and temperature which define the state, the fluctuations become too small to be important, reversibility disappears, the phenomenon becomes irreversible and the entropy increases. Apparently then entropy may decrease but does not because of the improbability that it will do so.

The second law of thermodynamics has many aspects. It is in the first place a denial of the *possibility* of a natural process attended by a decrease in

entropy. It is also in a sense a confession of our limitations. If like Maxwell's demon we could handle individual particles, we could cause processes to reverse and entropy to decrease. The law does not say that decrease of entropy is highly improbable but rather emphatically denies the possibility. If we admit a residue of probability even though it is extremely small, we must admit the possibility of decrease of entropy where by the law we deny it. The writer is inclined to the view that the decrease is indeed impossible, not highly improbable—and that the law is correctly stated in terms of the impossible. How then may we retain probability in our scheme of things and deny the possibility of an event which will decrease the entropy? Perhaps we may pass from improbable to impossible over the same bridge which Boltzmann built from reversibility to irreversibility via the mean value. When the number of events becomes large enough and the fluctuations relatively small enough, *i.e.*, when a mean is attained and preserved, certain configurations become impossible and not simply improbable.

If the theory of probability may be applied without restriction or limitation we must admit the possibility of any event which may have a probability assigned to it no matter how small. On the other hand if we accept the second law of thermodynamics in the form in which it is usually stated we must deny the possibility of certain operations which according to modern theory are merely highly improbable.

Of course it may be that highly improbable events like those already mentioned have occurred but, because of their momentary character or their location in distant parts of the universe, have escaped notice. Or they may occur at any time—not necessarily in the distant future—and strange phenomena may take place. On the other hand, the application of probability may be limited and the second law may reign in undisputed sway over a domain in which these strange phenomena are impossible. The latter view seems more reasonable.

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